From the Department of Medical Epidemiology and Biostatistics
Karolinska Institutet, Stockholm, Sweden

CAUSES AND CONSEQUENCES OF STRESS AND TOBACCO EXPOSURE IN UTERO ON BIRTH SIZE, ASTHMA AND ACADEMIC ACHIEVEMENT

Cecilia Lundholm

Stockholm 2021

All previously published papers are open access.
Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-086-5

Causes and consequences of stress and tobacco exposure in utero on birth size, asthma and academic achievement

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Cecilia Lundholm

The thesis will be defended in public at Atrium, Nobels väg 12 B, Solna,
Friday March 26th 2021 at 09:00 am

Principal Supervisor:
Professor Catarina Almqvist Malmros
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Co-supervisor(s):
Professor Henrik Larsson
Örebro University
School of Medical Sciences

Professor Göran Pershagen
Karolinska Institutet
Institute of Environmental Medicine
Unit of Environmental Epidemiology

Professor Brian M. D'Onofrio
Indiana University Bloomington
Department of Psychological and Brain Sciences

Opponent:
Associate Professor Liesbeth Duijts
University Medical Center Rotterdam
Erasmus MC
Department of Pediatrics

Examination Board:
Professor Mikael Norman
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Paediatrics

Professor Marie Eriksson
Umeå University
Umeå School of Business, Economics and Statistics
Department of Statistics

Associate Professor Linnéa Hedman
Umeå University
Department of Public Health and Clinical Medicine
Division of Sustainable Health

ABSTRACT

The objective of this thesis was to further the understanding of the consequences of stress and smoking during pregnancy, the causes of intrauterine growth and asthma as well as the consequences of asthma on school performance and to explore explanations of observed associations.

In Study I we investigated the associations between four measures of subjective distress and cortisol levels in early and late pregnancy and perinatal outcomes. All estimated associations were small and most statistically non-significant. However, for birth weight by gestational age the estimated associations with subjective distress were statistically significant, indicating higher birth weight with higher distress. Results were similar in early and late pregnancy.

In Study II we explored an intergenerational effect of smoking during pregnancy (SDP) on the grandchildren's intrauterine growth. The grandchildren of maternal grandmothers who smoked during pregnancy had a slightly higher risk of being large for gestational age, which may be partly explained by a higher obesity rate among their daughters (grandchild's mother). In contrast, the grandchildren of paternal grandmothers who smoked during pregnancy had slightly higher risk of being small for gestational age, which may be partly explained by a higher SDP rate among their daughters-in-law (grandchild's mother). Sensitivity analysis with regard to unmeasured confounding indicated that this may explain the associations.

In Study III, we explored the association between tobacco use during pregnancy and offspring asthma/wheeze and the potential role of nicotine and familial factors such as genes and environment shared within the family. We found that SDP was associated with a higher risk of asthma/wheeze in the first two years of life, but not at higher ages. Analysis of the association between oral snuff use in pregnancy, i.e. exposure to nicotine without combustion toxins, and asthma/wheeze indicated no clear association. The sibling comparisons showed lower estimates. Taken together this indicates that the role of nicotine in the association between SDP and asthma/wheeze may be limited, while familial factors seem important.

In Study IV we examined if adolescents with asthma in school Grades 7-8 and 9 perform worse in school compared to adolescents without asthma. Our results indicated that school performance of adolescents with asthma was somewhat better than among those without asthma, but also that asthma severity and control were important. Adolescents with severe, but controlled asthma, performed somewhat better than those without asthma, while children with uncontrolled asthma performed somewhat worse. Sibling analyses indicated familial factors explained most associations, with the exception of an association between uncontrolled asthma in Grade 9 and slightly lower school performance.

In conclusion, considering other known detrimental effects of the risk factors investigated in this thesis, our findings of no or very modest associations, should not in any way be

interpreted as excuses for ignoring stress in pregnant women, continue smoking during pregnancy or give up the strife for asthma control in children and adolescents.

LIST OF SCIENTIFIC PAPERS

I. **Lundholm C**, Rejnö G, Brew BK, Smew A, Saltvedt S, Almqvist C. Associations between maternal distress, cortisol levels and perinatal outcomes. *Submitted*

II. **Lundholm C**, Öberg S, D'Onofrio BM, Larsson H, Brew BK, Almqvist C. The role of maternal obesity and smoking during pregnancy in the transgenerational association between grandmaternal smoking during pregnancy and grandchild's foetal growth. *Submitted*

III. **Lundholm C**, Gunnerbeck A, D'Onofrio BM, Larsson H, Pershagen G, Almqvist C. Smoking and snuff use in pregnancy and the risk of asthma and wheeze in pre-schoolchildren-A population-based register study. *Clinical & Experimental Allergy*. 2020, 50(5):597-608. doi: 10.1111/cea.13593.

IV. **Lundholm C**, Brew BK, D'Onofrio BM, Osvald EC, Larsson H, Almqvist C. Asthma and subsequent school performance at age 15-16 years: A Swedish population-based sibling control study. *Scientific Reports*, 2020, 10(1):7661. doi: 10.1038/s41598-020-64633-w.

CONTENTS

LIST OF ABBREVIATIONS

ADHD	Attention deficit hyperactivity disorder
ATC	Anatomic Therapeutic Chemical classification system
BL	Birth length
BMI	Body mass index
BW	Birth weight
CAR	Cortisol awakening response
CDE	Controlled direct effect
CES-D	Center for Epidemiologic Studies – Depression Scale
CI	Confidence interval
DAG	Directed acyclic graph
ETS	Environmental tobacco smoke
GA	Gestational age
GINA	The Global Initiative for Asthma
HBW	High birth weight (> 4500 grams)
HPA	Hypothalamic-pituitary-adrenal
HR	Hazard ratio
ICD	International classification of diseases
ICS	Inhaled corticosteroids
IUGR	Intrauterine growth restriction
LABA	Long-acting beta2-agonists
LBW	Low birth weight (< 2500 grams)
LGA	Large for gestational age
LISA	Longitudinal integration database for health insurance and labour market studies
LTRA	Leukotriene receptor agonists
MAESTRO	Maternal Asthma Events, Stress and Offspring
MBR	Medical Birth Register
MGR	Multi-Generation Register
NDE	Natural direct effect
NIE	Natural indirect effect

NPR	National Patient Register
OR	Odds ratio
OR_{adj}	Adjusted odds ratio
OR_{obs}	Observed odds ratio
PIN	Personal identity number
PSS	Perceived Stress Scale
SAM	Sympathetic-adreno-medullary
SABA	Short-acting beta2-agonists
SD	Standard deviation
SDP	Smoking during pregnancy
SEI	Socioeconomic index
SGA	Small for gestational age
SPDR	Swedish Prescribed Drug Register
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TPR	Total Population Register
USS	Upper secondary school
WHO	World Health Organization

1 INTRODUCTION

A pregnancy is the start of a new human life and a period filled with expectations for future family life, but also of worry about not giving the unborn child the optimal intrauterine environment and about the health of the unborn child. Many parents are aware of the possibility that life in utero may influence the child's health both at birth and later in life.

Worry, stress or depressive mood during the pregnancy may have adverse effects for both the foetus and the mother-to-be. One early sign of a child's wellbeing is its growth in utero, but it is also important for the child not to be born too early. Does mothers' distress during pregnancy affect the child's intrauterine growth or gestational age at birth?

Although parents-to-be want the best for their unborn child, it may be difficult to live up to all expectations and we see that some women are unable to refrain from habits that may hurt the child, such as consumption of tobacco and other drugs during the pregnancy. Researchers have found evidence of intrauterine life not only influencing the foetus and the newborn, but also health in childhood and adulthood. Smoking during pregnancy is known to affect the birth weight of the child, but could it also affect the intrauterine growth in the next generation? A disease that is believed to be influenced by the intrauterine environment is childhood asthma. Tobacco smoke exposure in utero in particular is believed to cause asthma, but the mechanism is not clear.

Asthma may be a life-threatening disease if untreated, but it may also have other more subtle consequences. Ability to perform well in school is important for the child's future and may be influenced by asthma symptoms. Thus, understanding the consequences of maternal distress and smoking during pregnancy as causes of reduced intrauterine growth, shorter gestational age, or asthma, as well as the consequences of childhood asthma is important for parents, children, teachers, healthcare professionals and society.

BACKGROUND

1.1 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

In 1986 David Barker studied rates of infant death in towns in Britain and mortality due to a number of causes in the same towns almost fifty years later. He saw a striking correlation between infant death and ischemic heart disease. Based on those results he formulated the hypothesis that early life nutrition was important for ischemic heart disease later in life [1]. Barker's hypothesis has been called the foetal or developmental programming hypothesis, and more lately, Developmental Origins of Health and Disease (DOHaD). The concept has developed to include not only early life nutrition, but all kinds of early life exposures, such as chemicals, tobacco smoke, light, stress, and microbiota [2].

There is a wide range of studies, both experimental and epidemiological, showing associations between early life exposures and health outcomes in later life. However, the mechanisms by which those early life exposures may influence outcomes later in life are unclear [3]. It could be due to adaptive changes that are valuable for the foetus/child in the short run, but which could cause dysfunction or morbidity later in life [4]. Some of these adaptive changes could appear through epigenetic modifications including DNA methylation, modification of histones and non-coding RNAs. They change the gene regulation without changing the DNA sequence and could explain how one genotype can cause different phenotypes [5]. However, it is also likely that at least some of the associations seen are due to other mechanisms or confounding.

1.2 STRESS AND DISTRESS

Hans Selye has been attributed the first description of stress, which was published in the 1930's [6]. He described it as 'the non-specific response of the body to any demand'. According to Lazarus' stress-coping model, stress is an environmental demand exceeding a person's ability to meet it [7]. There are two components of stress. The first part is a threat (stressor) and the second part is the reaction in the body in response to the stressor [7].This reaction comes from three biological systems that are related. The neuroendocrine systems involved in controlling the stress are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adreno-medullary (SAM) limb of the autonomic nervous system. When exposed to a stressor, the sensory systems of the brain evaluate the stressor in relation to the current situation and previous experiences. Then, if a challenge to homeostasis is detected, the autonomous nervous system is activated which makes the SAM release noradrenaline and adrenaline. Noradrenaline and adrenaline increase the blood volume pumped out by the heart and raise the blood pressure, moves blood from the gut and skin to the muscles and makes the liver release glucose into the blood. At the same time the HPA axis is activated, which causes a release of cortisol. This release of cortisol is necessary for the fight-or-flight response [8].

When quantifying stress, we can either measure the actual stressor, the psychological response to the stressor or the biological response to the stressor. Examples of stressors are stressful life events, job stress, marital stress, extreme temperatures or noise, which are

usually measured by questionnaires or physical measurements. The psychological response is the individual's subjective reactions to stressors and ability to cope [9]. Distress is a broad concept covering a wide range of subjective response to stressors, which may include depressive symptoms, anxiety and sleep problems [8]. Examples of questionnaires for measuring distress are the Perceived Stress Scale (PSS) [10], the Beck Depression Inventory [11], the Brief Stress and Coping Inventory [12] and Center for Epidemiologic Studies Depression Scale (CES-D) [13].

When using the biological approach, stress can be measured as biomarkers, which can be regarded as objective measures of stress [9]. A commonly measured stress biomarker is cortisol, which varies over the day. It increases the last hours before awakening, with a rather steep increase during the first 30 minutes, followed by a decrease over the day and early night [14]. Cortisol can be measured in saliva, blood, urine or hair. Samples in both saliva and blood capture the daily pattern and the correlation between the two is usually high with a time lag of no more than 1-2 minutes [15].

Although cortisol is a measure of stress response, there are mixed results from studies on the correlation between subjective measures of stress and cortisol. The reasons for this may be small studies, variation in timing of measurements, differences in study populations and stress measures [16].

1.2.1 Distress in pregnancy

Distress during pregnancy is believed to cause preterm labour, low birth weight and pregnancy-induced hypertension [17,18]. It may also increase the risk of neuropsychological developmental delay, obesity and asthma in the offspring [17,18].

The mechanism by which maternal distress would affect the foetus is not clear [19]. An early observation was a decreased blood flow to the foetus [20], although it has never been replicated [21]. Another suggestion is mediation through foetal exposure to elevated cortisol levels. Although the placenta works as a barrier which protects the foetus from high levels of cortisol, some cortisol crosses the placenta. About 80–90% of the cortisol is inactivated by an enzyme (11β-hydroxisteroid dehydrogenase type-2), and converted to biologically inactive cortisone when crossing the placenta [19,22]. As a result, the cortisol levels of the foetus are only 10-20% of those of the mother. Nevertheless, 30-40% of the variation in the foetal cortisol levels may be explained by variation in maternal cortisol levels [19,22].

Although mean levels of cortisol increases as pregnancy progresses [23], it has been shown that the maternal HPA axis is downregulated during the course of the pregnancy such that the cortisol response to acute stressors and awakening decreases [24,25]. It is also possible that the foetus could be exposed to elevated levels of cortisol, despite cortisol levels not being elevated in the mother, as a result of this downregulation of the HPA axis. This could happen, due to changes in the placental function, causing more cortisol passing through the placenta, when the mother is stressed [26]. As for results on associations between distress measures and cortisol levels in general, statistically significant associations have been found in

pregnant women in several studies [27-36], while others found none [37-41]. In studies finding statistically significant associations a general pattern has been lower morning cortisol levels, flatter diurnal decline and higher evening cortisol levels with higher levels of distress.

In preparation for Study 1, we identified a knowledge gap. *To our knowledge there were no studies which had investigated the correlation between subjective distress and cortisol measures at different time points during pregnancy, as a way to explore the down regulation of the HPA axis.*

1.3 TOBACCO USAGE

Researchers have identified more than 5300 chemical compounds in cigarette smoke, including nicotine, benzene, carbon monoxide, nitric oxide, polycyclic aromatic hydrocarbons (PAHs), lead and cadmium [42,43]. According to the International Agency for Research on Cancer, seventy of the compounds in cigarette smoke have been deemed carcinogenic [43]. However, tobacco smokers are not only at increased risk of cancer; tobacco smoke is also a major risk factor for cardiovascular and pulmonary diseases and adverse pregnancy outcomes [42].

Swedish moist oral snuff is a tobacco product which is placed under the upper lip. As it is not burnt, snuff users are not exposed to the chemical compounds that come from combustion of the tobacco, but they are often exposed to high levels of nicotine [44].

1.3.1 Tobacco usage during pregnancy

Tobacco smoking during pregnancy (SDP) has declined in many countries in the past decades [45,46]. In Sweden the prevalence of SDP has decreased from 31% in 1983 to 5.6% in 2013 (Figure 1) [47]. However, there are large differences between groups, e.g. among pregnant teenagers the prevalence of SDP is still >20% at the first antenatal care visit, while it is below 4% among women of 30 years and above [47]. Other factors that have been identified as associated with SDP are low socioeconomic status, parity, marital status, birth country/ethnicity and passive smoking [48-50]. Data from the Swedish National Board of Health and Welfare on snuff use during pregnancy shows a fairly constant usage of 1.0-1.4% from year 2000 to 2013 (Figure 1).

Despite the reduction in SDP, it is still an important exposure with adverse effects on the pregnant woman and her foetus [45,46]. It has been found to be associated with a number of complications during the pregnancy, including placenta previa, placental abruption, and premature rupture of membranes [51]. Several studies have also found evidence of adverse effects in the offspring. Low birth weight and small for gestational age (SGA) are among those and it is believed that the exposure causes growth retardation as well as premature birth. There is also consistent evidence of associations with perinatal death, cognitive and neurobehavioral deficits as well as cleft lip and/or palate in the offspring [42]. Moreover, reviews and meta-analyses of epidemiological studies have shown associations between SDP and worse lung function and respiratory disorders in the offspring [52-55]. As with SDP,

Swedish moist oral snuff use in pregnancy has been linked to increased risk of stillbirth [56,57], preterm birth [58,59,60], lower birth weight [59,61], small for gestational age [62], neonatal apnoea [63] and oral cleft malformation [64], but unlike SDP, also to an increased risk of preeclampsia [59].

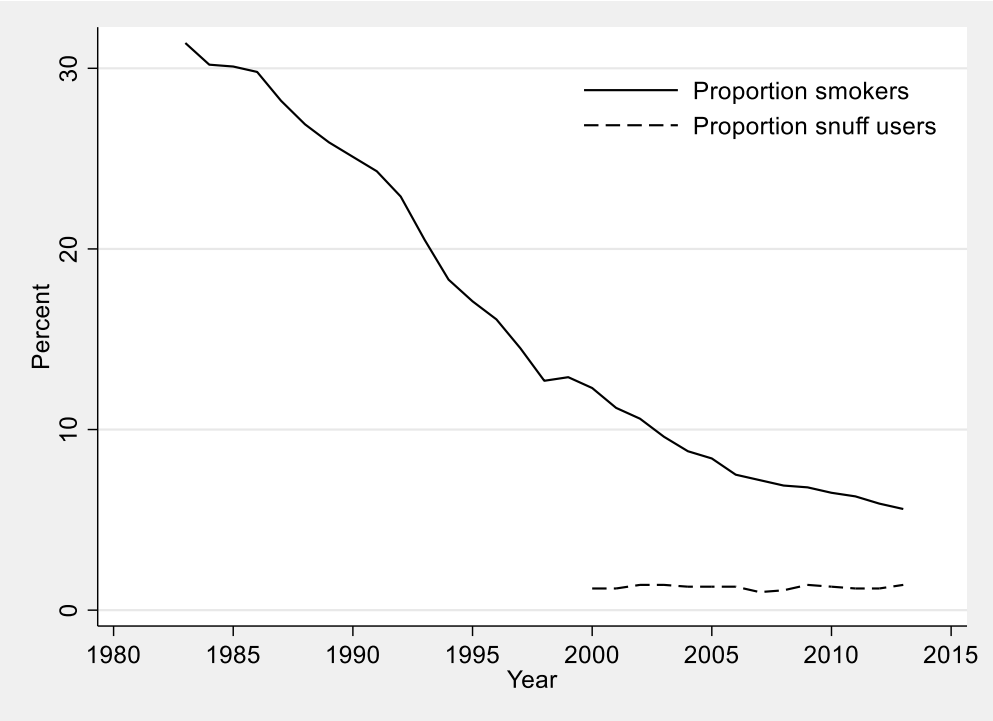

Figure 1. Prevalence of smoking and oral snuff use during pregnancy at first antenatal care visit 1983-2013, based on a report from the Swedish National Board of Health and Welfare [47].

1.4 PERINATAL OUTCOMES

1.4.1 Gestational age at birth

Gestational age at birth is based on an estimate of when conception took place, a time point which can be estimated based on either the mother's reported date of last menstrual period or ultrasound measurement of the size of the foetus. Since the 1980's almost all pregnancies in Sweden are dated using ultrasound measurements, usually in weeks 16-20 [47]. Preterm birth has been defined by the World Health Organisation (WHO) as birth before 37 full weeks from the first day of the woman's last menstrual period [65]. However, the cut off at 37 weeks is arbitrary and research has shown that also early term (weeks 37-38) infants are at increased risk of some adverse outcomes compared to infants born weeks 39-40 [66-68]. A wide range of risk factors for premature birth have been identified, among others low or high maternal age, nulliparity, short inter-pregnancy interval, smoking, recreational or illicit drug use, male foetus, and a wide range of maternal, placental, uterine and foetal conditions [69]. Complications due to premature birth has been deemed the leading cause of neonatal death, accounting for 34% of all neonatal deaths worldwide [70]. Preterm birth is also considered a

risk factor for worse neurodevelopmental outcomes, higher risk of hospital admission, behavioural and learning problems [69].

1.4.2 Intrauterine growth and birth size

Two important components influence birth weight (BW) and birth length (BL) – pregnancy duration and intrauterine growth. Birth weight and low birth weight (LBW) are often used as proxies for intrauterine growth and intrauterine growth restriction (IUGR). IUGR is defined as a restraint in growth such that the child does not reach its genetic growth potential. Small for gestational age (SGA) is a closer proxy for IUGR. Although many of the infants classified as SGA have suffered from IUGR, constitutionally small children may also be classified as SGA, as the measure does not take heritability and the growth trajectory into account [71].

IUGR has been considered important as the affected infants are at increased risk of neonatal mortality and early infections [71]. Furthermore, there is evidence of an increased risk of childhood disorders such as asthma and neurodevelopmental disorders, and impaired health in adulthood e.g. psychiatric disorders, hypertension and insulin resistance among individuals born with IUGR, although some of those associations are disputed [71-73].

There are many factors influencing intrauterine growth – maternal, placental and foetal factors. Examples of maternal factors are vascular disorders, pulmonary disease, anaemia and smoking [71,74,75]. Genes, multiple gestation and intrauterine infections are among the foetal factors that are thought to influence intrauterine growth [71,75].

1.4.3 Distress during pregnancy and perinatal outcomes

Distress during pregnancy has been associated with negative perinatal outcomes such as giving birth prematurely or to a child with LBW. A meta-analysis on antenatal stress, measured by stress questionnaires or stressful life-events (exposure to bereavement and hurricanes), and its association with LBW and preterm birth showed associations with odds ratios (OR) in the range 1.42-1.98 [76].

In a large systematic review focusing on antenatal depression and the associations with preterm birth, gestational age (GA), birth weight or LBW, the authors reviewed 50 reports on preterm birth or GA and deemed the results inconclusive, while they concluded the results for birth weight or LBW (33 reports) were indicative of an association [77]. In contrast, a meta-analysis study, based on 4-15 reports, found a statistically significant 37% increased odds of premature delivery if the mother was depressed, compared to mothers who were not depressed, while estimates for GA, LBW and birth weight were all small and statistically non-significant [78]. Likewise, Grote *et al* [79] found depression to be associated with preterm birth and LBW, but less so for intrauterine growth restriction. However, their results also showed that associations were stronger in developing countries compared to the United States and Europe. They also found stronger associations in studies of lower quality.

Anxiety was found to be associated with prematurity (pooled ORs: 1.41-1.54) and LBW (pooled ORs: 1.76-1.80) in two meta-analysis studies [80,81], while one of the studies also showed associations with GA, birth weight and SGA [81].

Studies on the association between sleep quality and perinatal outcomes are rarer. A meta-analysis study on sleep, premature birth and LBW included four small studies (338 study participants in total) on the association with premature birth and two studies (56 participants) on the association with SGA, both including studies with quality issues [82]. They found a statistically significant two-fold odds of preterm birth in women with poor sleep quality and 50% increased odds for SGA, which was not statistically significant.

The relation between maternal salivary cortisol in pregnancy and birth weight has been studied with different cortisol measures and varying findings [22]. The rate of increase in cortisol levels during the first 30 minutes after awakening (cortisol awakening response, CAR) in early (<27 weeks) pregnancy has been found to be associated with subsequent lower BW and BL in the offspring [37]. Likewise, higher morning cortisol values has shown statistically significant associations with SGA [83], and lower BW [83,84], but also with higher BW [38]. Statistically significant associations has also been found between higher evening cortisol levels and SGA [36], shorter gestation [28], lower BL [27] and lower BW [28,36,84]. Flatter diurnal slope has been associated with shorter gestation [36], and lower birth weight if measured in late pregnancy [28,38,85,86], but not in early pregnancy [85]. Cortisol levels at any time of the day has been found to be associated with shorter gestation [87,88] and lower BW [88]. Associations have further been found between larger area under the cortisol curve and lower birth weight [40,84,89].

One small study of 80 pregnant women, 40 with major depressive disorder and 40 without, has provided estimates of associations between depression symptom scales, urinary cortisol levels and the perinatal outcomes GA and foetal growth and the role of cortisol in the associations between depression symptoms and the perinatal outcomes. Their results indicated that cortisol levels explained 90% of the negative association between depressive symptoms and GA, and 45% of the association with foetal growth [90].

Although one study has shown an important role of cortisol in the association between depression symptoms and perinatal outcomes, it is still unclear if cortisol levels are mediating the associations between distress and offspring's birth size and gestational age at birth. This knowledge gap was identified in preparation for Study I.

1.4.4 Grandmother's smoking during pregnancy and grandchild's birth weight

There has been an interest, not only in the potential effect of maternal smoking during pregnancy on the exposed child, but also in the potential effect it may have on the next generation. One reason is that maternal SDP may reduce BW in the offspring, and a positive association between maternal and offspring BW has been demonstrated repeatedly [91]. Thus, one potential causal pathway through which grandmaternal SDP could affect the BW

of the grandchild is if the mother's BW is reduced due to SDP, then her own children may also suffer from reduced BW. However, the results from a twin study indicated that the association between mother's and child's BWs is not causal but rather driven by genetic similarity [75], suggesting a causal pathway via maternal BW would be unlikely. Lower BW in the grandchild could also be the result if there were a pathway mediated by maternal SDP. Another potential pathway stems from evidence that LBW would increase the risk of obesity [92], which in turn could affect BW in the next generation offspring. Epigenetic differences caused by SDP could give rise to pathways like those mentioned, but also to other pathways. From some recent studies there is evidence of such epigenetic changes, in which newborns exposed to tobacco smoke in utero had a different methylation pattern than newborns of non-smoking mothers [93-95] and the differences prevailed until school age [93,94]. It is still unknown whether those differences will last into adulthood.

In 2003 a study by Hyppönen et al was published on grandmaternal smoking during her pregnancy and the BW of the grandchild [96]. They assumed a reduction in BW in the grandchild since there is a negative association between maternal SDP and offspring BW and a positive association between a mother's own BW and that of her child. The BW of the grandchildren of smoking grandmothers was lower in unadjusted analyses, but not in the adjusted analyses. A study by Pembrey *et al* focused on the BW of the children whose mothers smoked during pregnancy and the potential effect of the grandmothers' smoking during pregnancy [97]. Once again, there was a reduced BW, although the difference was not significant in the adjusted analyses. The same research group also published a study focusing on the offspring of non-smoking mothers. In that study, they observed that boys with smoking grandmothers generally had higher BW. The difference was smaller among girls. [98]. In both those studies, they investigated both maternal and paternal grandmother's SDP, with some difference in point estimates, but no significant effect difference between the two. Rillamas-Sun *et al* divided their study population in two groups depending on the birth year of the grandmother [99]. They saw different patterns in the two groups. If the grandmother was born before 1929, the difference in BW between grandchildren of smoking and non-smoking grandmothers was small and not statistically significant. If the grandmother was born in 1929-1945, the BW of the grandchildren was statistically significantly higher if the grandmother smoked during pregnancy.

In a study on maternal smoking during pregnancy, an interaction with grandmaternal smoking during pregnancy was included. They saw that the association between maternal smoking and BW was more pronounced if the grandmother had also smoked during her pregnancy. They made no direct comparison between grandchildren of smoking vs non-smoking grandmothers [100]. However, from their results, it is possible to derive point estimates of the mean BWs among grandchildren of smoking grandmothers and among those of non-smoking grandmothers. The mean for the grandchildren of smoking grandmothers was slightly higher than that of non-smoking grandmothers.

In a recent publication on this topic, there was also a higher BW in grandchildren of grandmothers who smoked, although not statistically significant [101]. Thus, several studies have shown a higher BW in grandchildren of grandmothers smoking during pregnancy compared to those of non-smoking grandmothers [98-101]. Two studies found a lower mean BW in the grandchildren of the grandmothers who smoked during pregnancy compared to those of the non-smoking grandmothers, although that was not seen in adjusted analyses [96,97].

In many of the studies on grandmother's smoking during pregnancy and her grandchildren's birth size, there is a notion of mediation by maternal factors, but as far as we are aware there are no studies clearly distinguishing between confounders and mediators. One exception was a study focused on grandmaternal body mass index (BMI) and the association with the grandchildren's BW. They included estimates of the association between grandmaternal SDP and BW of the grandchildren with maternal SDP as a potential explanation for the association [102]. *Thus, based on previous research it is difficult to draw conclusions about potential causal pathways in an intergenerational effect of grandmaternal SDP on the BW of their grandchildren, a knowledge gap that was identified in preparation for Study II.*

1.5 CHILDHOOD ASTHMA

Asthma is one of the most common chronic diseases in childhood, with the worldwide prevalence estimated to 14% among 13-14 year olds [103]. In a Swedish study on 7-8 years old children the proportion who had ever been diagnosed with asthma by a physician was 7.4%, while 13% had experienced wheezing the past twelve months [104].

The disease affects the bronchial tubes in the lungs, where there is a chronic inflammation. The most typical symptom is wheezing. Other symptoms are breathlessness, chest tightness and cough. Triggers can be airborne allergens, respiratory infections, exercise or cold weather.

Risk factors for asthma which have been suggested in the literature include family history of asthma, LBW, damp home environment, obesity (both maternal and own), environmental tobacco smoke, low socioeconomic status of the parents, parity, maternal SDP and respiratory infections [73,105-109].

There are two categories of asthma drugs, those used to prevent inflammation and exacerbations (controller medication) and those used to treat exacerbations (reliever medications). The first choice of controller medication is inhaled corticosteroids (ICS), which, if needed, can be combined with long-acting beta2-agonists (LABA) or leukotriene receptor agonists (LTRA). Short-acting beta2-agonists (SABA), reliever medication, are used to widen the airways in case of an exacerbation. The goal for the treatment is to reach asthma control. More recent advances in the treatment of severe asthma, where asthma control has been difficult to reach are the so called biological therapies, including omalizumab, mepolizumab, reslizumab, benralizumab and dupilumab [110].

The Global Initiative for Asthma (GINA) has issued stepwise treatment recommendations for increasing levels of severity with the goal of fully controlled disease [111]. The asthma is considered fully controlled if the child has asthma symptoms or need reliever medications no more than twice weekly, does not wake up at night due to asthma symptoms and experience no limitation with regard to activities [111]. According to the European Respiratory Society and American Thoracic Society Task Force a patient has severe asthma if he/she needs high dose ICS in combination with another controller medication, in order to achieve a controlled asthma or if the disease remains uncontrolled despite this treatment [112].

1.5.1 Tobacco use in pregnancy as risk factor for childhood asthma

Reviews and meta-analyses have shown associations between SDP and childhood respiratory disorders such as wheezing and asthma [52-55]. To some extent, a causal effect of SDP on respiratory disorders are supported by mechanistic animal studies. Sekhon *et al* [113] found nicotine receptors in the lungs of monkey foetuses, which suggest a mechanism involving nicotine as the chemical compound involved. They have also shown in an experimental study that the foetuses of monkeys infused with nicotine, in amounts comparable to heavy smoking, during pregnancy, had lower lung weight and lung volume than foetuses of unexposed monkeys. The exposed monkey babies also performed worse on some lung function measures [114]. *In preparation for Study III we identified a knowledge gap. As far as we were aware, there had been no studies yet on nicotine exposure of the foetus due to use of products high in nicotine with low levels of other toxins, such as Swedish moist oral snuff, and offspring asthma.*

Tobacco smoking and asthma also share potential risk factors, e.g. low socioeconomic status and parity. Thus confounding could be an alternative explanation for the associations seen. Apart from adjustment for potential known confounders in epidemiological studies, one study has addressed the issue of causality by using sibling design. They studied the association between SDP and a number of offspring somatic and behavioural outcomes, including asthma at 7 years of age. They had a birth cohort with >50 000 children born 1959-1974, including 7 400 sibling groups of 2-6 siblings. The only statistically significant association they found for asthma was with heavy smoking during pregnancy (>20 cigarettes/day) in the full cohort. However, the corresponding point estimate in the sibling analysis was higher, but not statistically significant, indicating a potential lack of power [115]. *A larger sibling study could potentially provide more insight in the issue of confounding from family factors. This knowledge gap was addressed in the second part of the aim of Study III.*

1.6 SCHOOL PERFORMANCE

Apart from limiting future educational prospects, school performance in childhood or adolescence is associated with general health [116,117], mental health [118,119] and mortality [116,120] in adulthood.

In younger children school performance is often measured as abilities in reading, writing and mathematics. In older children or adolescents school grades, test scores, school attendance, drop out and eligibility to further education are commonly used educational outcomes.

Among the factors thought to influence school performance we have parental socioeconomic factors [121,122], parental involvement and expectations [123,124], male gender [122,125], prematurity [126], the child's own personality and attitude [127], general cognitive ability [124] and neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) [128,129].

Until recently (2018), most children in Sweden began school the year they became seven years old and it was compulsory to attend school for nine years (Grade 9). After compulsory school, most students apply to upper secondary school (USS). Admittance to USS is based on the grades from Grade 9. To be eligible for USS the student needs to pass all three core subjects – Swedish, mathematics and English. National tests in those core subjects are given in Grade 9, which are important when teachers grade their students.

1.6.1 Childhood asthma and school performance

Several studies have shown that compared to children with asthma, in particular severe asthma, are more absent from school, which could affect their academic achievements and grades [130-132]. Other factors, that may have a detrimental effect on the schooling of children with asthma, are acute exacerbations, sleep disturbances or side effects from asthma medication. Sometimes parents or teachers believe their children with asthma are too vulnerable to take part in particular school activities [133,134]. Further, a child's school performance may be affected by stress induced by having a chronic illness [133].

The conclusions from two reviews were that the association between asthma and school performance is weak or non-existent [130,132]. A more recent meta-analysis showed that asthma was associated with lower cognitive function in several domains in childhood, including academic achievement, executive functioning and global intellect, compared to their healthy peers, [135]. According to a recent Swedish study, based on the BAMSE cohort, children with asthma had a higher risk of low school performance compared to children without asthma [136]. Considering that the factors in proposed pathways should be more prevalent in children with severe or uncontrolled asthma, studies on asthma and school performance should include those aspects. It is also well-established that the prevalence of ADHD is higher among children with asthma [137-139] and that ADHD is associated with poorer school performance [128]. Irani *et al* conclude that there is a need of research on the association between asthma and school performance taking aspects like asthma control and ADHD into account [135].

It is also well established that school performance of children from families with lower socioeconomic background is poorer compared to children from higher socioeconomic circumstances [121]. Furthermore, several researchers have found associations between family socioeconomic status and asthma [105,108,140]. *Taken together this highlights the*

risk of confounding by ADHD, socioeconomic status and other family factors, when estimating the association between asthma and academic achievement. This knowledge gap was addressed in Study IV.

1.7 EPIDEMIOLOGICAL METHODS

In medical research, the randomised controlled trial is the gold standard for drawing conclusions about causality. However, when investigating potential causes of disease, or effects of disease, a problem is that we can rarely randomise the exposures of interest, e.g. stress, smoking and asthma. Thus, we rely on observational data and consequently we are faced with the issue of confounding.

Using directed acyclic graphs (DAGs) is a way to get an overview of factors that may influence the associations of interest [141]. They can be used to guide the decision on what factors to adjust for, what not to adjust for and what unavoidable biasing pathways we have (e.g. unmeasured confounders). DAGs have been criticised for being too simplistic [142,143], thus care is needed when using them.

1.7.1 Family design in epidemiology

A common approach to deal with confounding has been to measure and adjust for potential confounders in regression models.

Another approach is to use quasi-experimental designs, i.e. not randomising individuals to exposure, but rather use designs that can help us rule out some alternative explanations. Family-based quasi-experimental designs utilise the fact that individuals within the same family share characteristics that could confound associations of interest [144]. The most obvious example is identical twins, who share the same genome as well as intrauterine environment and usually childhood environment to a large degree. By comparing outcomes in exposure discordant identical twin pairs, the so-called co-twin control design, we can rule out confounding factors shared within the pair. However, there are limitations to the co-twin control design. One problem is that they sometimes also share the exposure by default, e.g. intrauterine exposures, such as SDP and maternal stress. In such situations the twin pairs do not contribute with information for co-twin control estimates. Another common problem is lack of power, as identical twins are rather rare. Then sibling design is an option, as in the study on SDP and offspring asthma by Gilman *et al* [115]. Siblings do not share as much as identical twins, but they still share 50% of the segregating genes and much of the childhood environment.

When using sibling/twin comparison design, we automatically adjust for all shared confounders, no matter whether they are measured or not. It should be noticed that we also adjust for all shared factors on the causal pathway (mediators). This could be an issue if we aim to estimate the total effect of an exposure, i.e. the effect of the exposure irrespective of causal pathways. On the positive side is that it can be shown that we do not adjust for shared colliders [145].

It is also important to consider the effect of measurement error and non-shared confounders. Frisell *et al* [146] have shown that biases due to those issues may be more serious in sibling comparison (paired) analyses than in ordinary unpaired analyses. In particular, the attenuation of estimates due to measurement error will be larger than in unpaired analyses. They also show that if the correlation of the exposure within sibling pairs is larger than the correlation of the confounders, then the sibling comparison analysis will be more biased than the corresponding unpaired analyses.

An assumption made in sibling comparison designs is that there are no carry-over effects from one sibling to the next, such as the exposure or outcome in the first sibling affects the exposure or outcome and vice versa. Some types of carry-over effects are either unproblematic or can easily be dealt with, while others are more problematic. Some of the more problematic cases can be shown to lead to conservative estimates of the target parameter. However, in one situation – when the outcome in one sibling affects the exposure in another sibling – it is difficult, or impossible, to determine the direction of the bias [147].

In conclusion, sibling comparison design is a powerful tool for causal inference in observational studies. Nevertheless, it is important to acknowledge the limitations when drawing conclusions from this type of studies, in particular when we see no exposure effect.

1.7.2 Mediation analysis

After having identified exposure-outcome associations in epidemiological research, the question about potential causal pathways arise. Mediation analysis plays an important role in the investigation of such pathways. The aim is typically to explore the *total effect* of exposure on an outcome, the *indirect effect*, which is the part explained by the pathway via the mediator and the *direct effect*, which is the effect of the exposure on the outcome that is not explained by the mediator of interest. A common approach, often called the traditional approach, to this issue is to first adjust for potential confounders in e.g. logistic regression models, and then to additionally adjust for a potential mediator. With this approach, the regression coefficients from the latter model is typically interpreted as the direct effect, and if it does not change, compared to the model without the mediator, it is assumed that there is no mediation by those mediators. The indirect effect can also be calculated as the difference or ratio between the two regression coefficients for the exposure (difference method) [148,149].

It has been shown that this traditional approach is prone to be biased [148]. Under certain assumptions, this is a valid approach, although, when applied, assumptions made are rarely discussed. For a causal interpretation five assumptions regarding confounding are made: 1) control is made for all exposure-outcome confounding, 2) control is made for all mediator-outcome confounding, 3) control is made for all exposure-mediator confounding 4) there is no mediator-outcome confounder that is affected by the exposure and 5) there is no exposure-mediator interaction [150]. From Figure 2a, we can understand assumptions 1) – 3) as that we control for all C_1, C_2 and C_3 confounders, while assumption 4) is violated in Figure 2b, where there is an arrow between X and C_2.

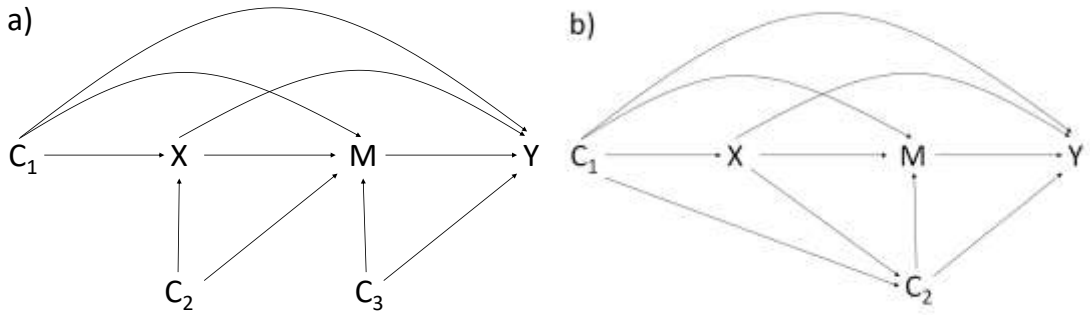

Figure 2. a) Relation between exposure X, mediator M, outcome Y and confounders C_1 and C_2. b) The same as figure a) but with a confounder C_2 being affected by the exposure X. Adapted from VanderWeele [149].

Moreover, if a logistic regression model is used with the difference method, the interpretation of differences between ORs is hampered by the non-collapsibility of the OR. Non-collapsibility means that the unadjusted (marginal) and adjusted (conditional) ORs are not directly comparable [151]. If the outcome is rare, this problem could be ignored, since, in that situation, the OR is approximately equal to the risk ratio [149]. However, in cases with a common outcome, the non-collapsibility makes it difficult to interpret a situation when the marginal and conditional ORs are approximately the same. That could be due to either no indirect effect or due to non-collapsibility.

By means of counterfactual reasoning, researchers within the field of causal inference, have highlighted the assumptions underlying the traditional approach and they have also developed methods that can deal with situations when some of these assumptions do not hold [149]. Within this framework controlled direct effects as well as natural direct and indirect effects can be defined [152].

The controlled direct effect (*CDE*) is defined as the effect of the exposure when the mediator is fixed at a certain value:

$$CDE = E[Y(1, M = m) - Y(0, M = m)]$$

Here *Y(x, M = m)* is the potential outcome if the exposure = *x* and the mediator = *m*. This is a measure that may be of interest for public health applications in situations when the mediator could be eliminated. However, there is no corresponding indirect effect.

The natural direct effect (*NDE*) is the effect the exposure would have had if we could set the mediator to what it naturally would have been for each individual if, for example, there was no exposure:

$$NDE = E[Y(1, M_0) - Y(0, M_0)]$$

M_0 = the value the mediator would have in the absence of exposure. If there is no interaction between exposure and mediator the *CDE* and the *NDE* will coincide.

The indirect effect (*NIE*) is the hypothetical difference in outcome if the exposure was fixed to a certain value, when the mediator has the value it would have had if each individual was exposed, compared to what the mediator would have been if they were unexposed.

$$NIE = E[Y(1, M_1) - Y(1, M_0)]$$

Here M_1 = the value the mediator would have if each individual was exposed.

2 RESEARCH AIMS

2.1 STUDY I

In study I we sought to estimate associations between measures of subjective distress and salivary cortisol, and potential differences in associations between early and late pregnancy, and their respective associations with perinatal outcomes. We also aimed to explore the role of cortisol as a potential mediator in an association between subjective distress and perinatal outcomes.

2.2 STUDY II

The aim of study II was to estimate the intergenerational association between grandmothers' SDP and the grandchildren's foetal growth (SGA and LGA). We also aimed to explore if an association could be explained by higher frequencies of SDP or obesity in the mothers, i.e. the role of maternal SDP and obesity as mediators in the association.

2.3 STUDY III

In study III we aimed to explore the role of nicotine and familial confounding in the association between tobacco use in pregnancy and offspring asthma. Here Swedish moist oral snuff use served as an indicator of exposure to nicotine without the combustion products from cigarette smoking. The role of familial confounding in the association between SDP and offspring asthma was explored using family design with comparison within families with both exposed and unexposed siblings.

2.4 STUDY IV

Our aim in study IV was to investigate if children with asthma in Grades 7-8 and Grade 9 have a worse school performance in Grade 9, in terms of grades, eligibility to upper secondary school, and results on national tests in English, mathematics, and Swedish, compared to children without asthma. We also aimed to explore the role of asthma severity, asthma control and familial factors in this association.

3 MATERIALS AND METHODS

In 2007 “The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement” [153] was published in several medical scientific journals, including The Lancet, Epidemiology and Journal of Internal Medicine in order to improve the reporting of observational studies, with more detailed information published in a subset of those journals [154]. Those guidelines have formed the basis in reporting of methods and results in all four studies.

3.1 STUDY DESIGNS

3.1.1 Study I

Study 1 was based on a clinical cohort, the Maternal Asthma Events, Stress and Offspring (MAESTRO) study of n = 1693 women. A range of factors, including stress, was evaluated at two occasions during pregnancy, first in connection to the first antenatal care visit, usually in gestational week 8-12 and then again in week 28-32. The women also provided saliva samples at both occasions. The women were followed up for the perinatal outcomes BW, BL, BW for gestational age and gestational age at birth.

3.1.2 Study II

Study II had an intergenerational design. Information on smoking during pregnancy as collected by the midwives was retrieved from the Medical Birth Register (MBR) for women (G1) who gave birth in 1982-1983. Their children, the parent generation (G2), were followed until 2012 for births of children of their own (G3). The cohort consisted of n = 55 118 children (G3) with information on maternal grandmother’s SDP and n = 37 506 children (G3) had information on paternal grandmother’s SDP.

3.1.3 Study III-IV

In study III and study IV we used both cohort design and sibling comparison design based on national registry data. In study III we included all children registered in the MBR between 1st July 2005 and 31st December 2012 with information on tobacco use during pregnancy n = 788 508. In study IV we included all children with grades from Grade 9 in the years 2008-2013, n = 570 595, and retrieved data on asthma diagnoses and asthma medication in Grades 7-8 and 9. In both studies we identified all differentially exposed sibling pairs within the cohorts for sibling comparisons.

3.2 MATERIALS

3.2.1 MAESTRO

The 1693 participants in the MAESTRO study were recruited in early pregnancy through eight antenatal care clinics in Stockholm between 2011 and 2014. At their first visit to the antenatal care the women were informed about the study by their midwives. If a woman agreed to participate she was asked to sign a written informed consent, provide biological

samples, answer questionnaires and a subsample of women were invited to do spirometry tests. The questionnaires included items on socioeconomic and obstetric background, stress, depression symptoms, worry, sleep and asthma. Blood samples were drawn at the antenatal care clinic and sampling kits for morning and evening saliva samples were mailed to the participants' home addresses in connection to recruitment and in week 28-32, along with sampling instructions. Due to funding and administrative reasons we randomly selected a subsample of participants for analysis of salivary cortisol. Women with both a questionnaire response and a corresponding saliva sample in either early or late pregnancy (or both) were eligible for selection to cortisol analysis.

3.2.2 National registers

For study II-IV we used Swedish national register data from the National Board of Health and Welfare and Statistics Sweden. Data from the different registries can be linked using the Swedish personal identity number (PIN), which is assigned to everybody with the intent to stay in Sweden for more than one year [155]. The PIN is unique for each resident. We used data from the Medical Birth Register, the National Patient Register and the Prescribed Drug Register held by the National Board of Health and Welfare. We also used data from the Total Population Register, the nationwide census of 1985, the Longitudinal integrated database for health insurance and labour market studies (LISA by Swedish acronym) and the Swedish National School Register, which are held by Statistics Sweden. Inclusion in those registers is compulsory for all Swedish residents by law.

3.2.2.1 Medical Birth Register (MBR)

The Medical Birth Register (MBR) started in 1973 and covers 96-99% of all live births in Sweden [156,157]. Pregnancies ending with spontaneous abortions before 23 weeks pregnancy (29 weeks until 2007) or induced abortions are not included. The data in the register are collected during pregnancy at the antenatal care clinics, which the vast majority of pregnant women in Sweden attend. Data around the birth of the child are recorded at obstetric units. Examples of data collected by the midwives at the antenatal care clinics are maternal weight and height, tobacco usage before and during pregnancy, family situation and diseases such as diabetes, lung disease and chronic kidney disease. The obstetric units collect data on the delivery, maternal diagnoses as well as anthropometric measures and health status of the newborn. Some information is available from 1973 and onwards, while other items has been added later, such as smoking during pregnancy which was added in 1982 and Swedish oral snuff use was added in 1999.

3.2.2.2 National Patient Register (NPR)

The National Patient Register (NPR) [158] started in 1964 and reached full national coverage for inpatient visits in 1987. In 1997 day surgery visits were added, while other specialist outpatient care was added in 2001, with approximately 80% coverage. Primary care visits are not included in the NPR. The register includes data on diagnoses according to the

International Classification of Diseases (ICD), non-pharmaceutical interventions, type of clinic, whether the visit was planned or unplanned and dates of admission and discharge.

3.2.2.3 The Swedish Prescribed Drug Register (SPDR)

The Swedish Prescribed Drug Register (SPDR) includes all prescribed drugs which have been dispensed at pharmacies in Sweden since July 1st 2005. In the register the active ingredients of the drugs are coded according to Anatomic Therapeutic Chemical classification system (ATC). The register also includes brand name, strength, amount dispensed, dosage form, dosage text and dates of prescription and dispense. Prescribed drugs which are not dispensed by the patient are not included in the register.

3.2.2.4 Total Population Register (TPR)

The Total Population Register (TPR) is a register of all Swedish residents from 1968 and onwards [159]. The TPR contains information on each resident's name, address, birthdate, biological sex, birth country, citizenship, marital status, migration within the country, immigration, emigration and death.

3.2.2.5 Censuses

Censuses were performed in Sweden in 1960 and every 5th year from 1970 to 1990 [160]. During the censuses each household had to fill out forms about all members of the household, including information on education, occupation and employment along with information about the living conditions in the household. Data from the censuses are available for researchers from Statistics Sweden.

3.2.2.6 Longitudinal integrated database for health insurance and labour market studies (LISA)

The LISA database started in 1990 as a register for research [160]. It is updated yearly and includes information on all residents that are 16 years or older on December 31st each year. Examples of data available from LISA are education, occupation, employment or unemployment, income, allowances, family relationship, sick leave, disability pension, social welfare and socioeconomic information. Most data in LISA is automatically retrieved from other registers, including the TPR.

3.2.2.7 Multi-generation Register (MGR)

The Multi-generation Register (MGR) [161] is a register containing information on each index person's biological parents and, where applicable, adoptive parents. Index persons are all residents who were born in 1932 or later and still alive in 1961. For 97% of all index persons born in Sweden the identity of the mother is available and the identity of the father is available for 95%. Missing identity of either parents is most common among index persons born in the 1930's as an important reason for missing information is that the parent died before the PIN was introduced in 1947 [161].

3.2.2.8 Swedish National School Register

Swedish National School Register includes children at all Swedish schools, except schools for children with intellectual disability. It contains subject grades, sum or mean of grades and information on eligibility to upper secondary school along with results on national tests in English, mathematics and Swedish all from Grade 9. Data is available since the school year 1987/1988 although the grading system has varied over time.

3.2.3 Variables

3.2.3.1 Stress and distress

Measures of stress and distress are mainly used in study I. For subjective distress we had four measures, all based on questionnaires.

The 10-items version of Cohen's Perceived Stress Scale (PSS-10), which measures the extent to which individuals have found their life situation stressful, during the past month [10,162,163]. Each item contributes to the total score by 0-4 points, giving a total score in the range 0-40 points. A higher score indicates higher stress level.

'*Worry'* was measured using a single item where the participants were asked to score their current degree of worry using a Likert scale from 0 to 10, with higher score indicating more worry.

The Center for Epidemiologic Studies Depression scale (CES-D) [13] was used to measure depressive symptoms in the past week, with scores ranging from 0 to 60 points, with higher scores reflecting more symptoms.

As a measure of '*Sleep quality'* we used a single question: "How do you usually sleep?" This question was answered using a Likert scale, 1 = very poorly and 5 = very well.

For objective stress we used salivary cortisol [164]. Instructions advised the participants to take a sample in the morning around 7 am, before having breakfast and brushing their teeth, and another sample in the eveing at approximately 9 pm. The actual time of sampling was to be written on a form accompanying the Salivette® tubes that were used for the samples and they were told to put the samples in the fridge immediately and mail them to Karolinska Institutet Biobank the next day. At the biobank the samples were centrifuged for two minutes before storage at –20°C. The samples were analyzed for cortisol levels, using the standardized CORT-CT2 radioimmunoassay kit (Cisbio Bioassays, Codolet, France). For statistical analyses we used the actual morning and evening cortisol levels with logarithmic transformation and the diurnal slope calculated as (morning level – evening level)/(time difference between samples in hours).

All measures of stress and distress were measured at recruitment in early pregnancy and all, except sleep quality, were repeated in late pregnancy (week 28-32).

3.2.3.2 Tobacco usage during pregnancy

Information on SDP and Swedish moist oral snuff use was collected from the MBR. Smoking information has been recorded in the MBR since 1982-1983. The pregnant women are asked about their tobacco habits at the first antenatal care visit (usually in week 8-12). They are asked about their current tobacco habits and, since 1999, about their habits three months prior to the visit. Since 1990, the women are asked again about their present tobacco habits at a visit around week 30-32. Smoking is registered as no smoking, 1-9 cigarettes/day and ≥ 10 cigarettes/day. Since 1999 tobacco habits also include Swedish moist oral snuff use (yes or no).

For study II we only had information about smoking at the time of the first antenatal care visit. For study III data was available for all tobacco habit questions which allowed us to use more refined definitions of tobacco use:

- '*Smoking in early pregnancy*' was defined as affirmative answers to smoking both at the time of the first antenatal care visit and 3 months earlier vs answering no to both questions.
- '*Still smoking in late pregnancy*' was defined as affirmative answer to smoking at all three occasions vs negative answers at all three time points.
- '*Smoking before or in very early pregnancy*' was defined as an affirmative answer to smoking 3 months before the first antenatal care visit, but negative answer to smoking at the time of the first visit vs negative answers to smoking at both those time points.
- '*Moist oral snuff use*' was defined as affirmative answers to snuff use both at the time of the first antenatal care visit and 3 months earlier vs negative answers regarding snuff use at both time points.

3.2.3.3 Perinatal outcomes

BW, BL, GA, SGA and LGA were all recorded at birth and retrieved from the MBR. For the vast majority (98%) of pregnant women in Sweden, foetal ultrasound is used for pregnancy dating of gestational age. If no ultrasound is done, date of last menstruation is used.

BW for gestational age (BW Z-score) as a continuous measure was calculated using BW, GA and sex based on reference curves for intra uterine growth by gestational age, which are based on foetal weights estimated by ultrasound and assuming a standard deviation (SD) of 12% of the mean [165]. The same reference curves are used for SGA and LGA in the MBR, where SGA is defined as a BW > 2 SD below the mean curve and LGA as BW > 2 SD above the mean curve, thus approximately corresponding to the 2.5^{th} and 97.5^{th} percentile, respectively.

We defined LBW in accordance with the WHO as a weight < 2 500 grams [65], while we applied a commonly used cut off for high birth weight (HBW), > 4 500 grams.

3.2.3.4 Childhood asthma

Our asthma variables were based on records of dispensed asthma medications in the SPDR and asthma diagnoses in the NPR in accordance with a validation study [166]. As a basis for all our asthma definitions we had the following three criteria:

1) ≥ 2 dispenses of asthma control medication, ICS (ATC: R03BA), LTRA (ATC: R03DC03) or combinations of ICS and LABA (ATC: R03AK) or
2) ≥ 3 dispenses of control medication and/or SABA (ATC: R03AC02–03, R03AC12–13) in the SPDR within 12 months or
3) ≥ 1 hospital visit with an asthma diagnosis recorded in the NPR (ICD-10: J45-J46).

For children below 4.5 years of age '*asthma ever*' was defined as fulfilling either criteria 1) or 2) in combination with criteria 3). For children above 4.5 years '*asthma ever*' was defined as fulfilling any of the three criteria. Time of onset was defined as the date of the first prescription of asthma medication or first asthma diagnosis in the NPR, whichever occurred first.

'*Current asthma*' at a certain age was defined as fulfilling the criteria for '*asthma ever*' in combination with having at least one dispense of asthma medication in the SPDR or one record with an asthma diagnosis in the NPR at that age.

For '*asthma in Grade 7-8*' and '*asthma in Grade 9*' we required any of the three asthma criteria to be fulfilled while being in Grade 7-8 and Grade 9, respectively, including time from July 1st the year the child started each grade until June 30th the year the child finished the grade.

Our definition of '*severe asthma*' was chosen to align as far as possible with the corresponding treatment steps of GINA [111]. To be defined as '*severe asthma*' an average daily dose of ICS corresponding to a medium dose or more for children ≥ 12 years old, as defined by GINA, was required, plus one (or more) other type of control medication. The average daily dose was defined as the total amount of ICS dispensed during the time interval divided by the length of the time interval in days. An asthma not deemed to be '*severe asthma*' was classified as '*mild/moderate asthma*'.

'*Uncontrolled asthma'* in Grade 7-8 was defined as having > 8 dispenses of SABA in total in the SPDR during those two years or at least one unplanned hospital visit with an asthma diagnosis recorded in the NPR in the same time, while '*uncontrolled asthma*' in Grade 9 was defined as having > 4 dispenses of SABA or an unplanned hospital visit with an asthma diagnosis during Grade 9.

'*Severe asthma*' and '*uncontrolled asthma*' were combined into an asthma severity and control variable with the categories *'no asthma', 'mild/moderate controlled', 'mild/moderate uncontrolled', 'severe controlled'* and *'severe uncontrolled'.*

3.2.3.5 School performance

'G*rade point sum*' from Grade 9 was the sum of all 16 subject grades. Each subject contributes with 0-20 points, giving a range of 0-320.

'Non-eligibility for USS' was defined as failing (0 points) in any of three core subjects: Swedish, Mathematics and English.

Results from national tests in the three core subjects was used as a measure of school performance that is sensitive to the student's performance in one particular day. The test results range from 0–20.

3.2.3.6 Other variables

Other variables that were retrieved from the MBR were birth year of the child, parity, maternal height and weight for calculation of BMI, maternal age at delivery and family situation (mother living with the father or not during pregnancy).

Family members (parents and siblings) were identified using the MGR and gender, birth date and birth country were retrieved from the TPR.

Asthma in each parent was defined in the same way as for a child above 4.5 years, although with the addition of diagnostic codes in the NPR from earlier ICD-versions (ICD-7:241, ICD-8:493, ICD-9:493).

Socioeconomic variables for the parents were retrieved from questionnaires for the study based on the MAESTRO cohort and from the censuses and LISA for the register-based studies. Thus, we retrieved immigrant status, education, work situation (employed/self-employed, student, other) and cohabitation with partner from the MAESTRO questionnaires. Immigrant status and education was collected in early pregnancy only, while information on work situation and cohabitation with partner were collected in both early and late pregnancy. For the register-based studies we retrieved socioeconomic index (SEI) from the census in 1985. Parental education and disposable income, at birth or at the time of the school start of the child, were retrieved from the LISA database.

ADHD was defined as having an ADHD diagnosis registered in the NPR (ICD-10: F90) or having dispensed ADHD medication (ATC codes: N06BA01, N06BA04, N06BA09) in the SPDR, in accordance with a validation study [167].

3.2.3.7 Variables by study

The variables used in each study as exposure, outcome, mediator and confounders are outlined in Table 1.

Table 1. Exposure, outcome, mediator and confounder variables by study

Study	Exposures and mediators	Outcomes	Confounders
1	PSS-10 Worry CES-D Sleep quality Morning cortisol Evening cortisol Diurnal slope	Birth weight Birth length Gestational age Birth weight Z-scores	Age at delivery BMI Parity Cohabit with partner Immigrant status Education Work situation
II	Exposure: Grandmaternal SPD Mediators: Maternal SDP Maternal BMI	Primary outcomes: SGA LGA Secondary outcomes: LBW HBW	Both grandmother (G1) and mother (G2): Age at delivery BMI Parity Family situation Grandmother (G1) only: Birth country Socioeconomic index Mother (G2) only: Education
III	Main exposures: 'Smoking in early pregnancy' 'Moist oral snuff use' Other exposures: 'Still smoking in late pregnancy' 'Smoking before or in very early pregnancy'	Asthma ever Asthma at ages 2, 3, 4, 5 and 6 years	Mother only: Age at delivery BMI Parity Family situation Mother and father: Birth country Asthma Education Disposable income Child: Birth year
IV	Asthma in Grade 7-8 Asthma in Grade 9 Asthma severity and control in Grade 7-8 Asthma severity and control in Grade 9	Grade point sum Non-eligibility for USS National test results: Swedish Mathematics English	Child: Gender Birth month ADHD Mother and father: Birth country Asthma Education Disposable income

3.3 STATISTICAL METHODS

In all four studies we used DAGs to select confounders (see Table 1). We presented results from both unadjusted models and models with adjustment for those selected confounders in accordance with the STROBE statement [153].

3.3.1 Study I

In study I all associations were estimated using linear regression analysis. The estimates were adjusted for confounders in accordance with Table 1, except the estimations of associations between subjective distress and cortisol, which were adjusted for a subset of the confounders – maternal age and BMI. As the actual time for taking the saliva sample was an important correlate of cortisol level in the sample, we adjusted for this in all analyses using cortisol data, despite not being identified as a confounder. We presented regression coefficients (β) and corresponding 95% confidence intervals (CI) for all associations, except those with log morning/evening cortisol levels as dependent variables. For those we presented the exponentiated regression coefficients, Exp(β), with corresponding 95% CI. Exp(β) is interpreted as the ratio between geometric mean cortisol levels corresponding to an increase of one unit in the explanatory variable. We also aimed to perform mediation analysis with cortisol levels as a mediator in the association between subjective distress measures and perinatal outcomes.

3.3.2 Study II

The first step of the analyses was to estimate all bivariate associations between SDP, underweight and obesity in one generation and SGA, LGA, SDP, underweight and obesity in the next generation. We also estimated the associations within generation two (G2), the mothers, between their own SGA/LGA and their SDP/underweight/obesity when being pregnant. All analyses were adjusted for grandmaternal (generation one, G1) confounders in Table 1, while associations between G2 and generation three (G3) were additionally adjusted for the G2 confounders in Table 1. All those associations were estimated using logistic regression. Logistic regression was also used to estimate the associations between grandmaternal (G1) SDP and SGA/LGA in the grandchild (G3) as an estimate of the *total effect*, adjusting for G1 confounders.

In mediation analyses we estimated *total effects*, *natural direct* and *indirect effects* (*NDE* and *NIE*) as defined in section 2.7.2, using the SAS macro *mediation* developed by Valeri *et al* [152]. Both mediators and outcome variables were modelled with the logit link, allowing for exposure-mediator interaction. The mediation analyses were adjusted for all G1 (exposure-outcome) and G2 (mediator-outcome) confounders in Table 1. We performed separate analyses for maternal and paternal grandmother's SDP.

As unmeasured confounding could be an issue, sensitivity analyses were performed using E-values. We calculated E-values for both *total effects* [168] and *indirect effects* [169] using the observed OR (OR_{obs}) as

$$\text{E-value} = OR_{obs} + \sqrt{OR_{obs} \times (OR_{obs} - 1)}$$

The E-value for the total effect is a measure of the strength, in terms of ORs, of the associations between the unmeasured confounder(s) and each of the exposure and outcome that would be required to give rise to an OR as that observed, if the true association was null. The E-value for the indirect effect is the corresponding associations required for the unmeasured confounder(s) of the mediator – outcome association, to give rise to the observed OR for the indirect effect, in a situation with no true mediation.

The secondary outcomes LBW and HBW were analysed in the same way, and the results were presented as supplemental material (Appendix II).

3.3.3 Study III

We analysed the association between tobacco use during pregnancy and offspring asthma/wheeze incidence, with flexible parametric models for time-to-event data with attained age as time scale, using the Stata package *stpm2* [170]. Follow-up started on the day the child was discharged from hospital after birth and ended at the date of asthma/wheeze onset, date of first emigration, date of death or 31 December 2013 for children with attained age <4.5 years and 31 December 2015 for older children, whichever came first. For the flexible parametric model, we used restricted cubic splines with six knots to model the baseline hazard. We allowed the hazard ratio (HR) to vary with time (age), again, using restricted cubic splines with six knots. Sensitivity analyses were performed with both higher and lower number of knots. We presented the results graphically with HR curves by age with 95% CI curves. As a comparison with the sibling analyses described below, we additionally used piecewise constant cox proportional hazards models allowing for different HRs in the age intervals 0-365 days, 366-730 days and ≥731 days. All models were adjusted for the confounders outlined in Table 1, modelled as time-varying effects in the same way as the exposure variables.

The associations between tobacco use during pregnancy and current asthma at the ages 2-6 years were analysed using logistic regression and adjusted for the same covariates as the time-to-event analyses.

We explored the role of unmeasured familial factors, using a sibling comparison approach. The sibling comparisons included all SDP exposure discordant full sibling pairs in the study population. Sibling comparisons account for all genetic environmental confounders and mediators, shared within the sibling pairs, including environmental tobacco smoke (ETS), if shared by the siblings. If the mother smoked in the pregnancy with a younger sibling, the older siblings may be exposed to ETS, which would constitute a carry-over effect in the sibling analyses. This was accounted for by including an interaction term between sibling order and exposure in all sibling analyses [147]. We used Cox proportional hazards model stratified on sibling pairs, assuming piecewise constant hazards in the age intervals 0-365 days, 366-730 days and ≥731 days, for asthma incidence and conditional logistic regression

for current asthma at ages 2 and 3 years. In the sibling analyses we used the covariates birth year, parity, maternal BMI and maternal age, as those would differ between siblings and robust standard errors were used to account for clustering of sibling pairs within families.

3.3.4 Study IV

In study IV we used linear regression analysis to estimate the associations between asthma and grade point sum and results of national test in the full cohort. Logistic regression was used for the associations between asthma and non-eligibility for USS. Residual distributions from the linear regression analyses were slightly skewed, we therefore used robust standard errors. The analyses in the full cohort included adjustment for the confounders in accordance with Table 1.

In the sibling comparisons, we included all full sibling pairs and we used fixed effects linear regression for the outcomes grade point sum and national test results, although only asthma discordant pairs contributed with information for the estimates of interest. Non-eligibility for USS was analysed using conditional logistic regression. As covariates we only used factors that are likely to differ between siblings, i.e. ADHD and family income the year the child started school. Cluster robust standard errors were used to account for clustering of multiple sibling pairs within families.

To help evaluating effect sizes from linear regression analyses, we calculated Cohen's $d = \beta/SD$, with β = regression coefficient and SD = standard deviation in the study population. Effect sizes can be categorized as small ($d \geq 0.20$), medium ($d \geq 0.50$) and large ($d \geq 0.80$) [171].

3.4 ETHICAL ASPECTS

In 1964 the World Medical Association agreed on ethical principles to be followed in research involving humans – the Helsinki Declaration [172]. Those ethical principles are there to protect the participants in research projects from injury – physical, mental or breach of their integrity. No scientific journals should publish research which does not adhere to the Helsinki Declaration. The document is not legally binding in itself, but it has been codified in regional or national legislation. The Swedish legislation incorporates principles of the Helsinki Declaration in the Ethical Review Act (2003:460), the Public Access and Secrecy Act (2009:400) and the Personal Data Act (1998:204), which was replaced by the EU directive the General Data Protection Regulation (GDPR) on May 25 2018. The Ethical Review Act states that research involving sensitive personal data, physical procedures in humans or biological samples from humans may only be performed if permission has been granted by the Swedish Ethical Review Authority (previously Regional Ethical Review Boards). The Ethical Review Authority weights the potential risk or harm for the study individual against the potential benefits of new knowledge. As a general rule the study participant has to be informed about the overarching study plan, aims, methods to be used, potential risks involved, who is responsible for the study, that participation is voluntary and that the participant can withdraw from the study at any time. Once informed the study

participant has to consent to participation in order to be included in the study (informed consent). This procedure was followed in study I, where biological samples were taken. In studies II-IV no physical procedures or other contact involving the participants were performed, therefore the Ethical Review Authority granted permission to perform the studies without informed consent. All four studies involved processing of sensitive personal data. For study I, data was retrieved by questionnaires, analysis of biological samples and linkage to register data, while studies II-IV was based on register data only. When ordering register data for a research project from the National Board of Health and Welfare and Statistics Sweden permission from the Ethical Review Authority is needed. The register holders make a secrecy examination, based on the ethical approval, where they determine if data can be disclosed. In particular they evaluate if all the ordered data is necessary to complete the research project. If the authorities decide they can disclose the data ordered, the data is pseudonymised, i.e. fields with personal identifiers are replaced by an artificial identifier to protect the integrity of the study participants, unless personal identification is necessary for the research project. After receiving the data for the studies in this thesis, it was uploaded in a database to which access is granted only to researchers needing the access to perform data management or analysis for the research projects and all data access is logged.

4 RESULTS

4.1 STUDY I

Of the n = 1693 women who were recruited we received complete answers for the questionnaire-based distress measures from n = 1055 to n = 1134 participants in early pregnancy and n = 880 (the same for all distress measures) in late pregnancy. We had cortisol measurements for n = 894 participants in early pregnancy and n = 682 in late pregnancy.

All the estimated associations between subjective measures of distress and cortisol levels were close to null and non-significant (Figure 2).

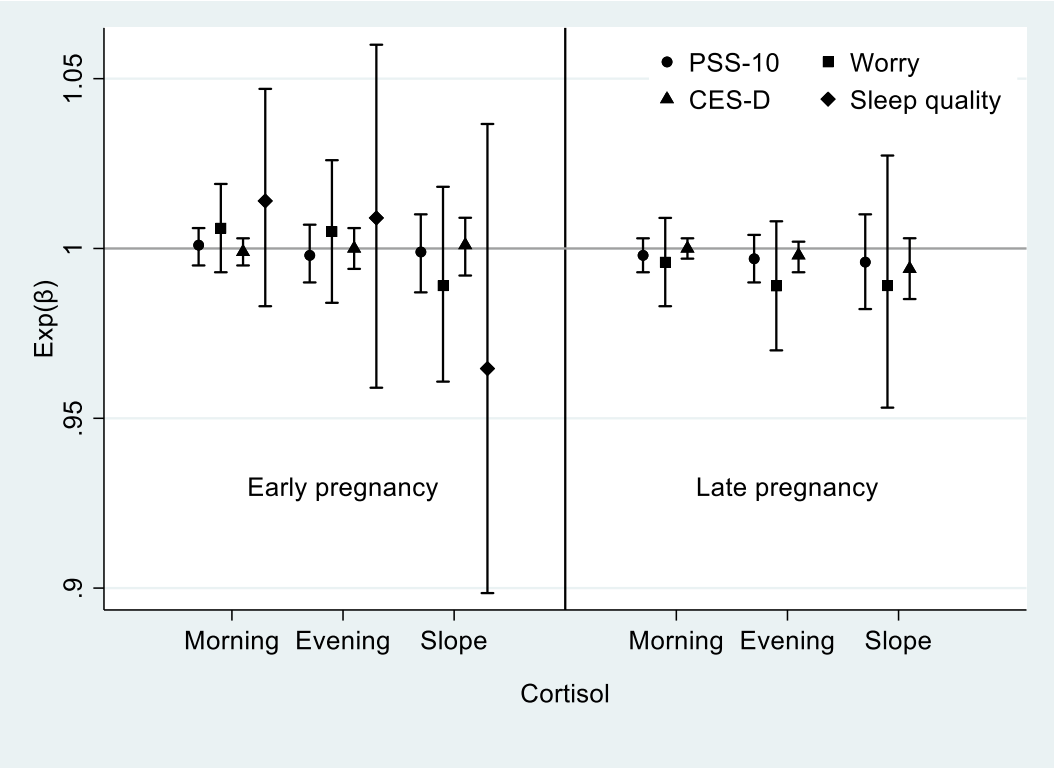

Figure 2. Regression coefficients with 95% confidence intervals for associations between subjective measures of distress and cortisol measures (morning level, evening level and diurnal slope), adjusted for confounders.

Associations between measures of distress in early and late pregnancy and perinatal outcomes are displayed in Figure 3. As with the associations between subjective distress and cortisol, most associations are close to zero and non-significant, no matter when in pregnancy distress was measured. The only exceptions are the estimates of the associations between most of the subjective measures of distress and BW Z-scores by gestational age and sex. Those estimates were also small, but statistically significant, indicating slightly higher BW Z-scores with higher levels of distress, and similar results were found for distress measured in early and late pregnancy. As an example, the children of women with PSS-10 levels corresponding to the third quartile = 18 would on average be 0.044 (95% CI: 0.002, 0.087) standard deviations

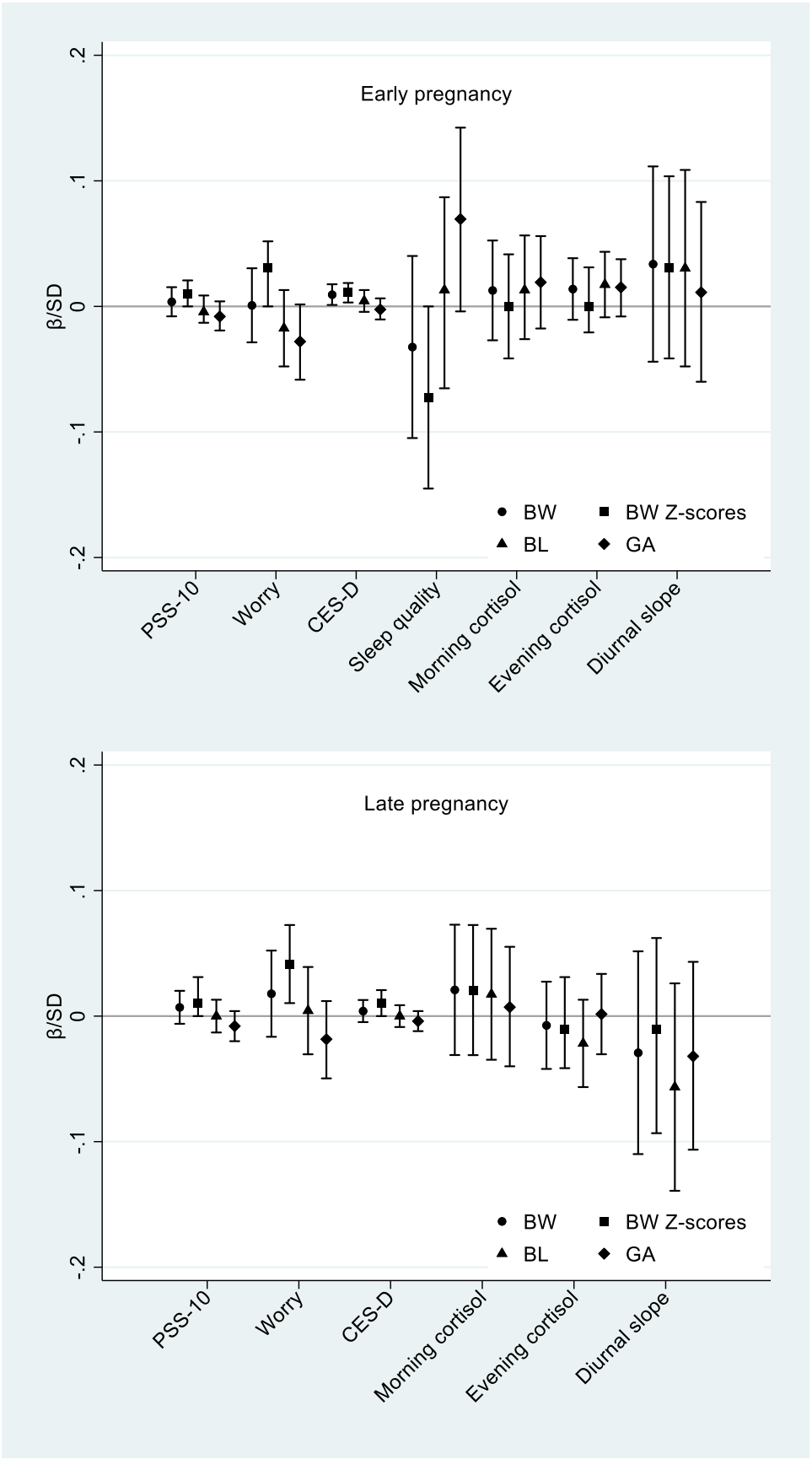

Figure 3. Regression coefficients and 95% confidence intervals estimating the association between subjective distress, salivary cortisol and perinatal outcomes, with the outcome in standard deviation units. BW = birth weight, BL = birth length, BW Z-scores = birth weight by gestational age and sex, GA = gestational age at birth.

heavier than the children of women having a stress level corresponding to the PSS-10 median = 14.

As the associations with cortisol levels were close to null, we could not proceed as planned with mediation analyses considering cortisol levels as mediator in the associations between subjective stress and perinatal outcomes. More details on Study I are found in Appendix I.

4.2 STUDY II

Children (G3) with maternal grandmothers (G1) who smoked during pregnancy were at higher risk of LGA than children with non-smoking maternal grandmothers, adjusted OR = 1.32 (95% CI: 1.16, 1.50), but not at higher risk of SGA (OR_{adj} = 0.92, 95% CI: 0.77, 1.09). In contrast, children with paternal grandmothers who smoked during pregnancy were at higher risk of SGA (OR_{adj} = 1.19, 95% CI: 1.00, 1.41) but not LGA (OR_{adj} = 1.05, 95% CI: 0.93, 1.20). More details regarding bivariate associations are found in Study II, Appendix II, tables 2, s1 and s2.

Considering that there was an increased risk of LGA, but not SGA, maternal (G2) obesity could potentially explain part of the association, but not maternal SDP, as SDP would increase the risk of SGA rather than LGA. Results from the mediation analyses are shown in Table 2. Those results indicated that the association between maternal grandmother's SDP and grandchild being born LGA was to a modest part explained by mediation by maternal (G2) obesity, with an OR = 1.05 (95% CI: 1.03, 1.06) for the *indirect effect*. In contrast, there was an increased risk of SGA in the grandchildren of paternal grandmothers who smoked during pregnancy, thus maternal (G2) SDP could potentially explain the association, while maternal obesity would be unlikely to explain the association. The mediation analysis showed that a small part of the association between paternal grandmother's SDP and the grandchild being born SGA may be explained by maternal (G2) SDP, with OR = 1.03 (95% CI: 1.01, 1.05) for the *indirect effect* (Table 2).

Table 2. Mediation analysis with maternal and paternal grandmother's (G1) smoking during pregnancy (SDP) as exposure, maternal (G2) obesity and SDP as mediators and grandchild's (G3) birthweight as outcome, including E-values for total and indirect effects.

Outcome (G3)	Mediator (G2)	Total effect OR[1] (95% CI)	Direct effect OR[1] (95% CI)	Indirect effect OR[1] (95% CI)	E-value	
					Total effect	Indirect effect
Exposure: Maternal grandmother's SDP (G1)						
LGA	Obesity	1.33 (1.17, 1.51)	1.27 (1.11, 1.44)	1.05 (1.03, 1.06)	1.98	1.27
Exposure: Paternal grandmother's SDP (G1)						
SGA	SDP	1.26 (1.02, 1.56)	1.23 (0.99, 1.52)	1.03 (1.01, 1.05)	1.84	1.21

SGA = small for gestational age, LGA = large for gestational age

[1] Odds ratios (OR) adjusted for grandmother's (G1) birth country, socioeconomic index, family situation, BMI, age at delivery and parity and maternal (G2) family situation, parity, age at delivery and education

The E-values showed that it would suffice with ORs around two for the associations of unmeasured confounders with both grandmaternal SDP and grandchild being born SGA or

LGA to give rise to our observed *total effects*. For the indirect effects the corresponding ORs for unmeasured mediator-outcome confounders were 1.21 and 1.27. More details on Study II are found in Appendix II.

4.3 STUDY III

In the cohort 6.2% of the mothers smoked in early pregnancy and 0.9% used Swedish moist oral snuff. The mean time of follow-up was 6.0 years, summing up to 4.7 million person-years. Results of asthma incidence allowing for time-varying HRs are displayed in Figure 4 for smoking in early pregnancy and snuff use in early pregnancy, both unadjusted and adjusted for the confounders outlined in Table 1. For smoking in early pregnancy, the HR increase from birth until a peak around five months of age. Around the age of twelve months the HRs increase again and reach a second peak around the age of 18 months. The estimates were clearly attenuated with adjustment for confounders, but the bimodality was still there. The association between maternal snuff use and offspring asthma incidence showed lower HRs and a less distinct pattern, although with a potential association around 1-2 years of age and, as with smoking, the estimates were attenuated when adjusting for confounders.

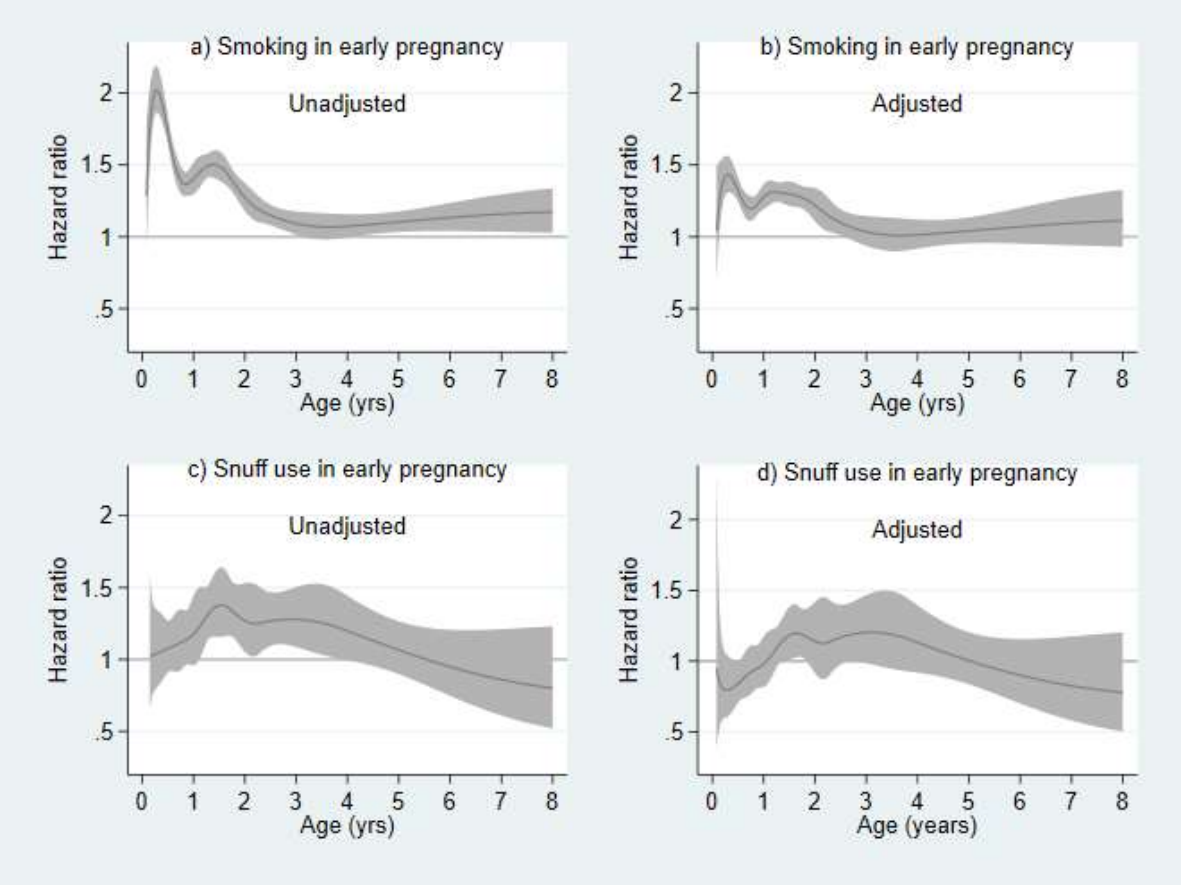

Figure 4. Hazard ratio curves with confidence intervals from flexible parametric models for the association between tobacco use in early pregnancy and offspring asthma.

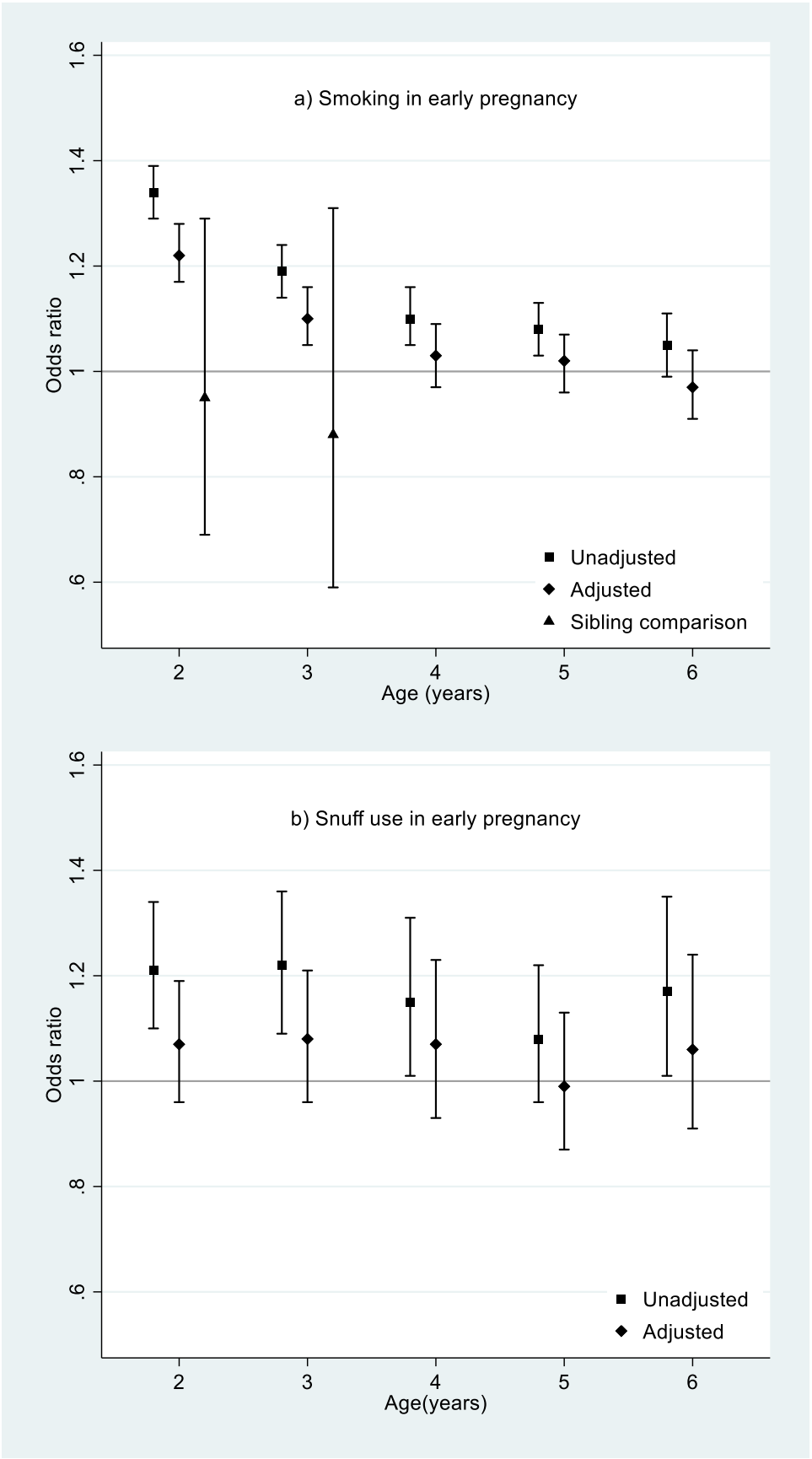

Figure 5. Associations between tobacco use in early pregnancy and current asthma at ages 2-6 years. Figure adapted from Lundholm *et al* [173].

Results for current asthma at ages two to six years showed a pattern of diminishing associations with smoking in early pregnancy with higher age, which were clearly attenuated when adjusted for confounders (Figure 5). For snuff use in early pregnancy the estimated associations were small and statistically non-significant at all ages in adjusted analyses. In the sibling analyses for smoking in early pregnancy the point estimates showed no increased risk of asthma, although the confidence intervals were wide.

The Cox proportional hazards regressions showed results in line with the flexible parametric models in terms of estimates and the results for the sibling analyses using Cox regression were similar to those from the sibling analyses for current asthma, more details of Study III [173] are found in Appendix III.

4.4 STUDY IV

There were n = 31 173 (5.5%) children with asthma in Grades 7–8 and n = 19 467 (3.4%) children had asthma in Grade 9. In the study population the mean grade point sum was 213 (SD = 62) and 8.9% were non-eligible to USS. Children with asthma in Grades 7-8 had on average 3.9 (95% CI: 3.3, 4.5) points better grade point sum than children without asthma, corresponding to a Cohen's d = 0.06, adjusted for confounders in accordance with Table 1, and they had a slightly lower odds for being non-eligible to USS (OR_{adj} = 0.84, 95% CI: 0.80, 0.88). When also adjusting for familial factors, by means of sibling comparisons, the corresponding difference in grade point sum was 0.4 points (95% CI: -0.6, 1.5) and OR for non-eligibility to USS was 0.99 (95% CI: 0.87, 1.12). For asthma in Grade 9 the results were similar for the associations with grade point sum and non-eligibility to USS [174].

When categorising asthma by severity and control we saw that children with controlled asthma had slightly better grades (Figure 6) and lower risk of being non-eligible to USS (Figure 7), for asthma in both Grades 7-8 and Grade 9, when adjusting for measured confounders, while children with *mild/moderate uncontrolled* asthma had slightly lower grade point sum and slightly higher risk of non-eligibility to USS. For controlled asthma estimates from sibling analyses were close to null, except for the associations with *severe controlled* asthma in Grades 7-8, indicating slightly better grades among those children. For *mild/moderate uncontrolled* asthma differences remained for asthma status measured in Grade 9, but not Grades 7-8.

Regarding associations with national test results a similar pattern was seen with slightly better results for children with severe controlled asthma and slightly worse for children with uncontrolled asthma compared to children without asthma. In sibling comparisons, estimates for controlled asthma were close to null, while those for uncontrolled asthma were slightly lower than for children without asthma, although statistically significant only for national test results in English for children with *mild/moderate uncontrolled* asthma compared to no asthma. More details of Study IV [174] are found in Appendix IV.

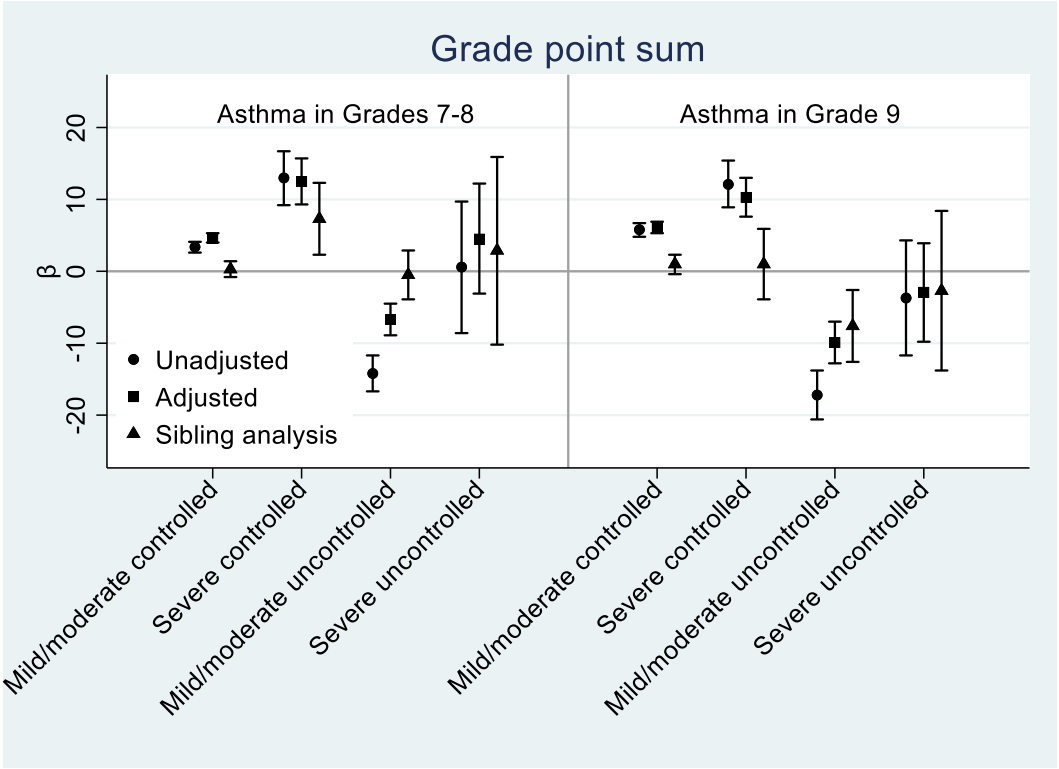

Figure 6. Regression coefficients with 95% CI for associations between asthma and grade point sum by severity and control in Grades 7-8 and Grade 9.

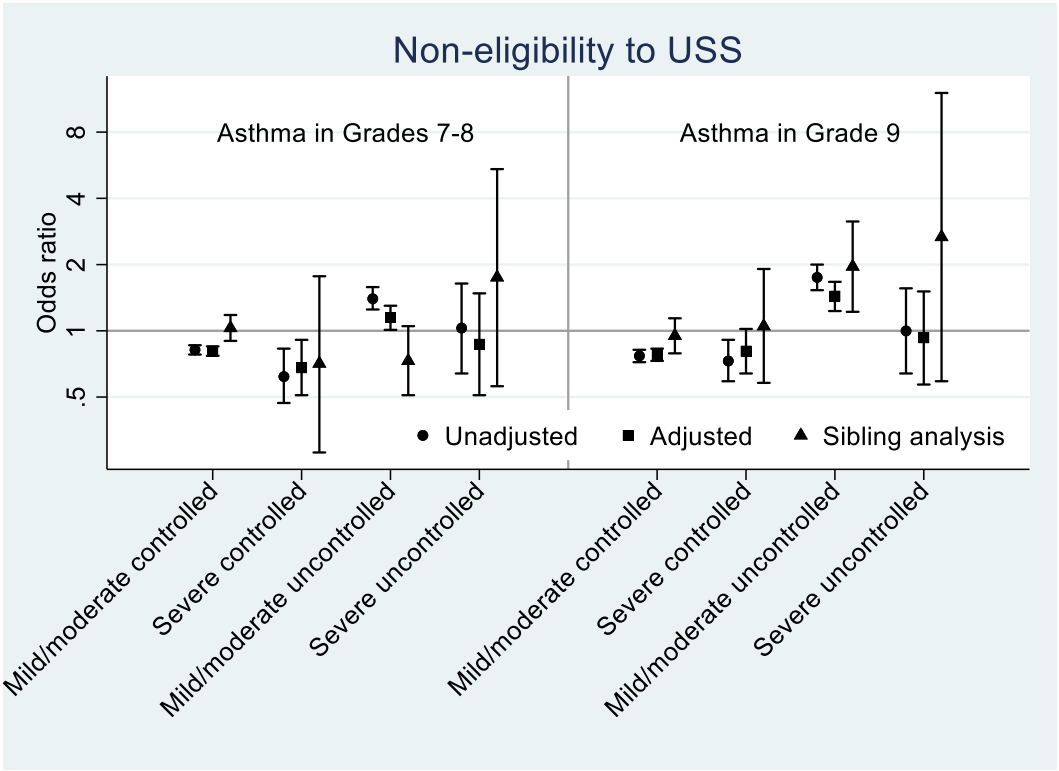

Figure 7. Odds ratios with 95% CI for associations between asthma and non-eligibility to USS by severity and control in Grades 7-8 and Grade 9.

5 DISCUSSION

5.1 METHODOLOGICAL CONSIDERATIONS

5.1.1 External validity or generalizability

External validity or generalizability to the target population is an important aspect of epidemiological research. This does not necessarily mean that the study population needs to be representative of the target population [175]. In Study I the mothers had lower BMI, higher education and lower SDP prevalence than the general population of pregnant women, indicating a more health conscious study population than the background population. However, our analyses were adjusted for socioeconomic factors, age and BMI, and the study population may bring the advantage of providing accurate information on health-related questions. In Study II, where we studied grandmaternal SDP, the mothers could be born no earlier than 1982 to have information on intrauterine exposure to SDP and follow-up for child births ended by year 2012. Consequently, the mothers (generation 2) had to give birth to a child by 30 years of age and as a result the findings may not be generalizable to a population of older mothers, if the biological pathways change with age. In Study III on tobacco use during pregnancy and offspring asthma/wheeze, our study population was selected from population-based registers, with the main restriction being availability of information on tobacco use, where we had 7% non-response. The mechanism leading to non-response is either the midwife failing to ask about tobacco usage or the pregnant woman refusing to answer, with the former being less problematic with regard to external validity. In Study IV on asthma and school performance the main obstacle to the external validity was the inclusion criteria requiring grades from Grade 9. However, complete absence of grades in the register is unlikely to be due to asthma.

5.1.2 Selection bias

Selection bias is related to external validity. If the study population is different from the target population, it may be a case of conditioning on a common effect of two factors that differ between the two populations. If one of those factors is the outcome or a cause of the outcome and the other factor is the exposure (or a cause of the exposure), selection bias occurs [176].

This could not be ruled out in Study I and II. In Study I we had a selection of women with higher education and lower BMI than the target population, where education is likely to influence stress levels and BMI is likely to influence intrauterine growth. Selection bias was also a potential problem in Study II, where the mothers, the second generation, could be no older than 30 years. Maternal age can be influenced by childhood socioeconomic status, which may also influence the grandmother's choice of SDP at the same time as it may be influenced by the mother's own socioeconomic status, which in turn may affect the grandchild's BW. In Study I we could adjust for education and BMI and in Study II we could adjust for socioeconomic status. However, we cannot completely preclude bias via other

unmeasured lifestyle factors not captured by education and BMI in Study I and socioeconomic status in Study II.

5.1.3 Misclassification and measurement error

Misclassification of exposure or outcome may bias association estimates. Misclassification can either be differential, i.e. differ between exposure groups or by outcome, or non-differential. Differential misclassification would lead to under- or overestimation of associations, depending on patterns of misclassification. Non-differential misclassification of the exposure would most likely lead to bias towards the null, while non-differential misclassification of the outcome would most often lead to lower precision in the estimates and thereby reduced power.

Two of our measures of distress in Study I, PSS-10 and CES-D, are psychometric scales considered to have high validity and are recommended for use in the general population and for pregnant women [13,162,163]. Worry and sleep quality have not been validated, but they all showed similar patterns of associations with the outcome as PSS-10 and CES-D. Salivary cortisol has the advantage of capturing the variations over the day, although that also carries the disadvantage of higher variability, as compared to cortisol measured in urine or hair. In order to reduce variability, study participants were asked to sample saliva at certain times in the morning and the evening. Although many followed the instructions some failed to do so, but the time of sampling was noted and the analyses were adjusted for the actual sampling time. Nevertheless, we could not exclude some attenuation of the estimates due to variation in sampling time.

Tobacco habits during pregnancy were reported by the pregnant women to the midwives when visiting the antenatal care clinics. As always with health hazardous behavior there is a risk of the women not answering truthfully. However, validation studies based on cotinine measurements with and without the women's knowledge has shown a good concordance between reported tobacco habits in the MBR and cotinine levels, with the exception of snuff usage in late pregnancy [44,177]. We therefore abstained from using the snuff information in late pregnancy in Study III.

The perinatal outcomes were all retrieved from the MBR and consequently measured and reported by health care professionals at the time of measurement, ensuring high quality data.

Our measures of asthma have been validated in a previous study, which indicated a high validity in the older children (> 4.5 years) for both asthma based on medication only and asthma based on doctor's diagnosis only, while the validity was lower for asthma based on medications only in the younger children [166]. In the younger group, we therefore required both medication and a doctor's diagnosis in the NPR to be classified as asthma.

School performance was retrieved from a national register, with data reported by schools, resulting in high quality data. Naturally the choice of grade point average, non-eligibility and results from national tests as measures of school performance could be discussed, as they may

be influenced by choice of school and parental factors. However, those are likely to be the measures of school performance from compulsory school with the highest impact on future educational prospects.

5.1.4 Confounding

Confounding is a central issue in all observational research. In Studies I and II we used the common approach of adjusting for measured confounders in regression models, consequently we could not preclude that our results are biased due to residual confounding. Indeed, in Study II where we complemented this approach by the use of E-values, we saw that the findings could potentially be explained by residual confounding. We could also see from bivariate analyses that a woman's SDP was associated with a 40% increased risk of her daughter-in-law being born SGA, i.e. a stronger association than that with her grandchild being born SGA, despite no biological relation. This can only be explained by confounding, including factors influencing choice of spouse. Taken together those results were strong indications of residual confounding explaining the paternal grandmother's SDP – grandchild SGA association in Study II. Similarly, we could suspect residual confounding also in the associations with maternal grandmother's SDP. In study III and IV we used sibling comparisons to account for unmeasured familial confounding that is shared between siblings, in addition to adjustment for measured confounders, as a way to partly address the issue of residual confounding. When accounting for those familial factors most of the estimates were close to null in both studies, indicating the importance of taking familial factors into account in studies like these, where socioeconomic and lifestyle factors may be important confounders.

5.1.5 Sibling design

Although sibling design has some strong advantages, it also comes with some limitations. One limitation that has already been mentioned in section 6.1.3 is the sensitivity to measurement error or misclassification of the exposure variable, which is a more serious problem in sibling design than in ordinary epidemiological designs. This usually leads to attenuation of the estimates and can therefore be a potential explanation for null findings, which ought to be considered.

Another limitation that may lead to underestimation of associations is the fact that we, in sibling designs, not only adjust for shared confounders, but also for shared mediators. What we estimate is in other words not the total effect, but the direct effect that is not explained by causal pathways via shared mediators. In Study III, where we estimated the association between SDP and asthma/wheeze, ETS in the household was a mediator that we adjusted for, as far as it was shared between siblings. Thus, we cannot be sure the attenuation was due to adjustment for shared confounders. The explanation may also be adjustment for ETS and other shared mediators.

Related to this is the loss of power in sibling designs, due to the fact that pairs only contribute to the effect estimates to the extent that they are discordant in the exposure. Sibling pairs

sharing exposure will only contribute indirectly and very little to the estimates. As a result, the confidence intervals for the sibling analyses were wide in Study III and likewise for the estimated associations between severe uncontrolled asthma and non-eligibility to USS in Study IV.

A fourth limitation stems from cross-over effects between siblings. This was also a potential issue in Study III. If a child gets asthma the parents should be advised to refrain from smoking. Consequently, if a child in a family gets asthma and the parents follow the recommendation to refrain from smoking this would affect the exposure of the foetus in a subsequent pregnancy. The assumption of no such cross-over effect is not testable. However, we estimated the risk of SDP if an older sibling had asthma compared to if the older sibling did not have asthma. It turned out that the mothers of the children with asthma were actually more likely to smoke in the next pregnancy compared to the other mothers. There is likely to be residual confounding affecting this estimate, but at least it indicates that it is unlikely that there would be a strong tendency towards refraining from SDP when an older child has asthma. Although this is a potential health issue for children with asthma, it was reassuring for our analyses.

5.2 FINDINGS

5.2.1 Study I

In Study I we could not replicate previous findings of associations between salivary cortisol and subjective measures of distress and likewise between salivary cortisol and perinatal outcomes. We also found little evidence of associations between subjective measures of distress and perinatal outcomes, with the possible exception of associations between subjective measures of distress and BW by GA and sex (BW Z-scores). However, the associations were weak and the direction was contrary to much of the previous research [77,78,81,82]. Thus, our results indicate no negative influence of maternal stress on perinatal outcomes. As a consequence of those results no mediation analysis could be performed.

A question that arises is why we did not replicate previous findings. Regarding associations between subjective measures of distress and cortisol levels in pregnancy, statistically significant associations have been found in several [27-36], but not all [37-41] studies.

One reason why results differ between studies and between our study and many others, may be differences in study populations. This has been seen in meta-analyses on distress and perinatal outcomes. One meta-analysis study showed that associations were weaker in studies from the United States and “European social democracies” compared to developing countries [79] and another study found stronger associations among women of lower socioeconomic background [78]. There may also be differences between studies with and without adjustment for confounders and between studies of different quality [78,79,81].

Another reason for differences in results may be choice of distress measures. Although a meta-analysis study found evidence of an association between distress and perinatal

outcomes [76], the results may be different when focusing on one particular measure of distress. Thus, we have found seven studies, with sample sizes varying between 92 and 865, investigating the PSS-10 or PSS-14 at different stages of pregnancy, in relation to birth weight. Most of those found no statistically significant associations with birth weight, preterm birth, GA or SGA, with point estimates pointing in different directions [34,37,178-180], with the exception of one study finding an association with SGA [181] and one finding associations with both preterm birth and LBW [182].

Several studies have found significant results for associations between salivary cortisol and perinatal outcomes [27,28,36-38,40,83-89]. In a review of the literature on maternal antenatal cortisol levels and child outcomes, Zijlmans *et al* [22] noted that most statistically significant estimates showed the same direction. However, a majority of the included studies did not find statistically significant associations and those who did had not adjusted for confounders.

5.2.2 Study II

In Study II we saw that the grandchildren of maternal grandmothers who smoked during pregnancy had an approximately 30% higher risk of being born LGA. In contrast the grandchildren of paternal grandmothers who smoked during pregnancy had approximately 30% higher risk of being born SGA. A small part of the association with maternal grandmothers' SDP seemed to be explained by the daughters of the grandmothers who smoked being more likely to be obese, while a small part of the association with paternal grandmothers' SDP seemed to be explained by their daughters-in-law being more likely to smoke during pregnancy. However, the e-values were low, in particular for the *indirect effects*, which indicated that residual confounding may explain the associations.

Our results for the *total effects* are in line with most previous research on maternal grandmaternal SDP and offspring intrauterine growth [98-101], but not all [96,97]. Only two previous studies have published results for paternal grandmothers' SDP, with point estimates in opposite directions, depending on whether the mother smoked or not during pregnancy [97,98]. Our results for paternal grandmothers' SDP were in line with those for children of mothers who smoked [97]. The novelties with our study were the focus on SGA and LGA rather than BW, the mediation analysis and sensitivity analysis for unmeasured confounding. Those results could not be compared to previous research.

5.2.3 Study III

In Study III the results indicated an association between SDP and asthma/wheeze in the first two years of life and possibly an association between snuff use during pregnancy and asthma/wheeze in a time window around 1-2 years of age. However, the sibling comparisons, which account for all confounders and mediators that are shared within the sibling pairs, did not show an increased risk of asthma/wheeze for the siblings exposed to SDP.

Our results are in line with a meta-analysis study finding an association between SDP and early wheeze in the child [183]. In the same study they also found an association between

ETS and early wheeze, for both prenatal and postnatal ETS. Some studies have separated SDP only, maternal postnatal smoking only, and both SDP and postnatal smoking, with mixed results for the association with offspring asthma/wheeze. Three studies found stronger associations with SDP only, than for postnatal smoking only, but also compared to exposure to both SDP and postnatal smoking [52,184,185], while one study found no difference [186]. Other studies found ORs for asthma or wheeze that were higher for postnatal smoking than for SDP, with or without the other [187,188], while one study found somewhat higher estimates for SDP only compared to ETS with or without SDP [189]. In our study we did not have information on maternal postnatal smoking or other ETS. However, the sibling comparison accounts for environmental confounders and mediators that are shared by the siblings, including ETS if both siblings were exposed.

Although others have found an association between Swedish moist oral snuff use in pregnancy and neonatal apnea [63], suggesting an influence of intrauterine exposure to nicotine on the respiratory system, we found no consistent association between snuff use and asthma/wheeze. In combination with results from our sibling analyses on SDP, those results suggest a limited role of prenatal exposure to nicotine in the development of asthma/wheeze.

5.2.4 Study IV

In Study IV we investigated school performance in children with and without asthma and the role of asthma severity and control. We found that most differences were likely to be explained by genetics and family environment. However, children with severe but controlled asthma in Grades 7-8 may perform slightly better in school than children without asthma, while having uncontrolled asthma in Grade 9 may lead to worse school performance compared to children without asthma.

A majority of previous studies have shown lower school performance in children with asthma compared to children without [136,190-196]. Other studies have had null findings or showed better performance among the children with asthma [197-202]. One possible reason for diverging results could be differences in asthma phenotypes, as we saw different results by asthma severity and control. Another possible reason is that most studies have had a mix of children of different ages, from primary school children to adolescents in upper secondary school [190,191,193,196,198,200,201,203]. According to Tsakiri *et al* children with asthma had lower grades than their peers in elementary school, but not in middle school [196]. Two smaller Swedish studies have compared grades from Grade 9 by asthma status, with one finding that children with asthma had lower grades [136] and one finding no difference [197].

Similar to us, an American sibling study from 1992, including 101 asthma discordant sibling pairs, found no difference in reading, mathematics and composite achievement between the siblings [199].

6 CONCLUSIONS

From the four studies included in this thesis we could draw the following conclusions:

- Based on the MAESTRO cohort we found small and statistically non-significant associations between salivary cortisol measures, concurrent measures of subjective distress and the perinatal outcomes BW, BL, BW Z-scores and GA, with the exception of a small but statistically significant association between worse subjective distress and higher BW Z-scores. Timing of stress measurement did not seem to matter.
- We saw a somewhat increased risk of LGA among children whose maternal grandmothers smoked during pregnancy, with an indication of a small part of the association being explained by maternal obesity. Likewise, we found a slightly increased risk of SGA in children whose paternal grandmother smoked during pregnancy, with a small part possibly explained by their own mothers' SDP. However, sensitivity analyses indicated that most of the results could be due to residual confounding.
- We also found that children of mothers who smoked during pregnancy were at higher risk of asthma/wheeze in the first two years of life. Nicotine seemed to have a limited role in this association, while unmeasured confounding and mediating factors shared among siblings seemed to play an important role for the association.
- Children with severe asthma did not seem to have lower school performance than children without asthma, if the asthma was controlled. For children with uncontrolled asthma the picture was more complicated. Familial factors seem to explain the negative associations with asthma in Grades 7-8, but not Grade 9. However, we cannot rule out residual confounding by factors associated with both asthma treatment adherence and school performance.

The results of the four studies included in this thesis have mostly shown very modest associations or associations that were likely to be explained by residual confounding or confounding by familial factors rather than being causal. However, other studies have found maternal stress, worry, depressive symptoms, and bad sleep quality to be associated with negative outcomes for both the unborn child and the mother [17,18,22]. SDP is believed to cause many serious adverse health effects for both the mother and the unborn child [45,46,51]. If a child's asthma is uncontrolled the child may experience worse respiratory problems in the future, be unable to participate in activities as other children and in the worst case suffer a life-threatening asthma exacerbation. Thus, the results of the studies of this thesis should not in any way be interpreted as excuses for ignoring stress in pregnant women, continue smoking during pregnancy or give up the strife for asthma control in children and adolescents.

7 FUTURE PERSPECTIVES

Although we found very weak evidence of an association between distress, cortisol levels and perinatal outcomes, in contrast to many other studies. Further research is needed to fully understand the effects of distress on the foetus. Are there certain types of distress that are associated with perinatal outcomes? What is the biological mechanism? If prolonged stress is more important than temporary stress, cortisol measurements taken from hair which reflects cortisol levels over longer periods could be a better mediator candidate than salivary cortisol [204,205]. Hair cortisol also has the advantage of allowing for tracing back in time and moreover, the sampling procedure is easy and non-invasive. Potential mediators are also other stress hormones, e.g. corticotrophin-releasing hormone or adrenocorticotrophic hormone and maternal factors that are causes or consequences of distress.

The intergenerational effects of SDP on intrauterine growth seem to be small, but may merit continued elucidation to better understand the potential dangers of tobacco smoke exposure. Considering the potential influence by unmeasured confounding, family-based designs may further the understanding of the underlying mechanisms.

Electronic cigarettes are a new form of nicotine exposure and the prevalence of regular use among young adults has been estimated to 3-4% in Sweden [206] and the United States [207]. Considering that some women are likely to continue vaping during pregnancy, the role of prenatal exposure to electronic cigarettes is an important area of research. However, in absence of vaping information in registers, such as the MBR, large cohort studies would be necessary to study potential risks for the foetus. In Study III, we used Swedish moist oral snuff to study nicotine exposure in utero. Nicotine is an important toxin from electronic cigarettes, although not the only one [208], thus snuff could be a feasible exposure alternative to electronic cigarette use during pregnancy in epidemiological research.

Although our results from Study IV were reassuring regarding negative effects from asthma on school performance, we could not rule out a potentially detrimental effect of uncontrolled asthma. It would therefore be valuable to better understand if the estimates were confounded by factors not shared by siblings. In particular it would be valuable to study how personal characteristics influence teenagers' treatment adherence and whether those factors affect school performance. Examples of characteristics that could be of interest are symptoms of psychiatric or neurodevelopmental disorders, forgetfulness, sensitivity to peer pressure and organisational skills [209].

8 ACKNOWLEDGEMENTS

The last year of my PhD-studies has been like no other year due to the Covid-19, mostly working from home and, with few exceptions, only seeing supervisors, colleagues, research collaborators and all the friendly faces at the department as small stamps on the computer screen. It has never been clearer to me how much it means to me to meet you all in person.

Catarina Almqvist Malmros, my main supervisor and friend, we had already worked together for nine years when I asked if you would consider being my PhD supervisor. It was an easy choice, which I have never regretted. I appreciate your honesty as scientist, your empathy, encouragement, but also your ability to push when needed.

Göran Pershagen, co-supervisor, apart from contributing with your expert knowledge in the tobacco exposure and asthma, I have very much appreciated your encouragement and engagement in my doctoral studies. Your praise of my abilities has boosted my confidence, something I will carry with me far beyond my PhD.

Henrik Larsson, co-supervisor, thank you for always making me feel that you are available for help and support. I admire your curiosity and analytical mind, which makes it fun and awarding to work with you, and I truly hope for future collaborations.

Brian D'Onofrio, co-supervisor, after seeing your critical, but constructive, comments on manuscripts that we both co-authored, I was convinced that I wanted you as a co-supervisor. I wanted a supervisor and co-author who would challenge me and ask the important questions that helps improve the quality of research, and you did. Thank you!

Paul Dickman, my nearest boss, you are the best boss one could have. You are a boss with a big heart, always supportive, available and trusting my ability. I particularly want to thank you for repeatedly nudging me to take up my PhD-studies again. Without you it would not have happened.

My co-authors: **Bronwyn Haasdyk Brew**, apart from being an encouraging and cheering friend, you also have an analytical mind that makes methodological discussions a pure joy. **Gustaf Rejnö**, thank you for all your hard work with the MAESTRO cohort and for teaching me so much about pregnancies and obstetrics. **Awad Smew**, your positive attitude and critical mind make you pleasure to work with. **Sissel Saltvedt**, your involvement in the MAESTRO study have been instrumental. **Sara Öberg**, thank you for being such a thorough and engaged co-author. **Anna Gunnerbeck**, apart from knowing all that is worth knowing about Swedish snuff, your energy and laughter are truly contagious. **Emma Caffrey Osvald**, working with you never gets boring and your knowledge in respiratory paediatrics and socioeconomics has been invaluable.

Andreas Jangmo, **Chen Wang**, and **Emma Caffrey Osvald**, thank you for agreeing to be on my pre-dissertation committee! **Patrik Magnusson**, thank you for accepting the role as chair at my thesis defense!

Kristina Johnell, **Kamila Czene**, **Per Hall** and **Paul Lichtenstein**, past and present heads of the Department of Medical Epidemiology and Biostatistics (MEB) during my PhD-studies, thank you for making MEB such a special work place.

All the participants of the MAESTRO cohort deserves a big thank you, without you there would not be a MAESTRO study. I am also grateful to all the research staff within the project – **Eva Warghammar**, **Malena Kjellén**, **Jessica Pege**, **Marlene Stratmann**, **Michael Broms**, **Aki Tuuliainen**, **Staffan Bergh** and **Jose Tapia** – for their invaluable contributions, and to all midwifes and other staff at the participating antenatal care clinics.

There are two groups at MEB that are particularly close to my heart, the research group in paediatric epidemiology and the applied biostatistics (ABS) group. **Anna Hedman**, **Anna Skarin Nordenvall**, **Chen Wang**, **Hanna Borgström**, **Lottie Philips**, **Marianne Bonnert**, **Mwenya Mubanga**, **Samuel Rhedin,** and **Tong Gong** you all make the atmosphere in the paediatric epidemiology group friendly and warm. It is an absolute joy to work with you!

Not only being a PhD student, but also an applied biostatistician means I throughout my PhD-studies also have had the pleasure to work with the ABS group. **Agnieszka Szwajda**, **Alexander Ploner**, **Anna Johansson**, **Arvid Sjölander**, **Hannah Bowers**, **Henric Winell**, **Li Yin**, **Michael Sachs**, **Mikael Franko Andersson**, **Paul Lambert**, **Ralf Kuja-Halkola**, **Robert Karlsson**, **Bjorn Roelstraete**, **Abid Lashari**, and **Mark Clements**, you have all helped me staying sane during those years. I really long to meet you IRL again!

The Biostat group makes MEB a dream for any biostatistician. I can't name all of you, but you all contribute to making MEB a fantastic work place.

Marie Jansson, without you admin would be a pain. Your eagerness to provide expert help in any administrative obstacle is invaluable. And you always do it with a smile! I'm also grateful for the support from past and present study directors, **Amelie Plymoth** and **Paul Lichtenstein. Camilla Ahlqvist** and **Alessandra Nanni**, thank you for all your help with education administration. **Sofia Anderberg**, **Sara Tiste**, and **Björn Gidlund**, thank you for helping out with HR issues. A big thank to **MEB IT** for smooth help with all IT issues. I'm also grateful for the support from our AC **Agneta Häggström** for wise advice.

All **MEB friends** it is impossible to name each and every one, but you all contribute to MEB's very special work environment.

My late **father**, coming from a working class background without the opportunity to university education, you made it clear that you expected us to make use of the educational opportunities we had. Dear **Mother**, when mentioning potential educations, you clearly told me to aim higher and not to waste talent. Now I have probably reached higher than any of you expected.

My brother **Jonas**, thank you for always being there when I need you and for believing in me. When I gave you the date of my dissertation, your spontaneous question was – "So what's

next, professor?" That is you in a nut shell. My niece and nephew **Kajsa** and **Eric**, I hope this can inspire you to aim high!

My dear parents-in-law **Siv** and **Gunnar**, thank you for your support and for the summer vacations in Grundsund, away from work and research.

Oscar, from now on I hope I will rarely say that I'm sorry I need to work, when you want to play or talk. **Johan**, the love of my life and my soul mate, thank you for always being there, for always caring, for cheering me up by fooling around, for being a shoulder to lean on and for practical help with everything from computer issues to statistical problems. Without you by my side this would have been much harder.

We acknowledge financial support from the Swedish Research Council (grant no 2018-02640) and through the Swedish Initiative for Research on Microdata in the Social And Medical Sciences (SIMSAM) framework grant no. 340-2013-5867, the Strategic Research Program in Epidemiology at Karolinska Institutet, grants provided by the Stockholm County Council (ALF-projects), the Swedish Research Council for Health, Working Life and Welfare, FORTE (grant no 2015-00289) and the Swedish Heart-Lung Foundation (grant no 20180512). The work of this thesis was also funded by Karolinska Institutet KID-funding.

We wish to thank the Biobank at Karolinska Institutet for professional biobank service and the Centre for Child Research, Stockholm South General Hospital, Stockholm for cortisol analyses.

9 REFERENCES

1. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet.* 1986;1(8489):1077-1081.

2. Suzuki K. The developing world of DOHaD. *J Dev Orig Health Dis.* 2017:1-4.

3. Barouki R, Melen E, Herceg Z, et al. Epigenetics as a mechanism linking developmental exposures to long-term toxicity. *Environ Int.* 2018;114:77-86.

4. Barouki R, Gluckman PD, Grandjean P, et al. Developmental origins of non-communicable disease: implications for research and public health. *Environ Health.* 2012;11:42.

5. Bianco-Miotto T, Craig JM, Gasser YP, et al. Epigenetics and DOHaD: from basics to birth and beyond. *J Dev Orig Health Dis.* 2017;8(5):513-519.

6. Selye H. A syndrome produced by diverse nocuous agents. *Nature.* 1936;138:32.

7. Lazarus RS. Coping theory and research: past, present, and future. *Psychosom Med.* 1993;55(3):234-247.

8. Fink G, (editor). *Stress: Concepts, Cognition, Emotion, and Behavior: Handbook of Stress Series Volume 1.* San Diego: Elsevier Science; 2016.

9. Kopp MS, Thege BK, Balog P, et al. Measures of stress in epidemiological research. *J Psychosom Res.* 2010;69(2):211-225.

10. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav.* 1983;24(4):385-396.

11. Steer RA, Ball R, Ranieri WF, et al. Further evidence for the construct validity of the Beck depression Inventory-II with psychiatric outpatients. *Psychol Rep.* 1997;80(2):443-446.

12. Rahe RH, Tolles RL. The Brief Stress and Coping Inventory: A useful stress management instrument. *Int J Stress Manage.* 2002;9(2):61-70.

13. Radloff LS. The CES-D scale: A self report depression scale for research in the general population. *Applied Psychological Measurements.* 1977;1(3):385-401.

14. Wilhelm I, Born J, Kudielka BM, et al. Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology.* 2007;32(4):358-366.

15. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology.* 1994;19(4):313-333.

16. Mikkelsen S, Forman JL, Fink S, et al. Prolonged perceived stress and saliva cortisol in a large cohort of Danish public service employees: cross-sectional and longitudinal associations. *Int Arch Occup Environ Health.* 2017;90(8):835-848.

17. Cardwell MS. Stress: pregnancy considerations. *Obstet Gynecol Surv.* 2013;68(2):119-129.

18. Vehmeijer FOL, Guxens M, Duijts L, et al. Maternal psychological distress during pregnancy and childhood health outcomes: a narrative review. *J Dev Orig Health Dis.* 2019;10(3):274-285.

19. Gitau R, Fisk NM, Glover V. Maternal stress in pregnancy and its effect on the human foetus: an overview of research findings. *Stress.* 2001;4(3):195-203.

20. Teixeira JM, Fisk NM, Glover V. Association between maternal anxiety in pregnancy and increased uterine artery resistance index: cohort based study. *BMJ.* 1999;318(7177):153-157.

21. Mendelson T, DiPietro JA, Costigan KA, et al. Associations of maternal psychological factors with umbilical and uterine blood flow. *J Psychosom Obstet Gynaecol.* 2011;32(1):3-9.

22. Zijlmans MA, Riksen-Walraven JM, de Weerth C. Associations between maternal prenatal cortisol concentrations and child outcomes: A systematic review. *Neurosci Biobehav Rev.* 2015;53:1-24.

23. Mastorakos G, Ilias I. Maternal hypothalamic-pituitary-adrenal axis in pregnancy and the postpartum period. Postpartum-related disorders. *Ann N Y Acad Sci.* 2000;900:95-106.

24. Entringer S, Buss C, Shirtcliff EA, et al. Attenuation of maternal psychophysiological stress responses and the maternal cortisol awakening response over the course of human pregnancy. *Stress.* 2010;13(3):258-268.

25. Kammerer M, Adams D, Castelberg Bv BV, et al. Pregnant women become insensitive to cold stress. *BMC Pregnancy Childbirth.* 2002;2(1):8.

26. O'Donnell K, O'Connor TG, Glover V. Prenatal stress and neurodevelopment of the child: focus on the HPA axis and role of the placenta. *Dev Neurosci.* 2009;31(4):285-292.

27. Bublitz MH, Bourjeily G, D'Angelo C, et al. Maternal Sleep Quality and Diurnal Cortisol Regulation Over Pregnancy. *Behav Sleep Med.* 2018;16(3):282-293.

28. Gilles M, Otto H, Wolf IAC, et al. Maternal hypothalamus-pituitary-adrenal (HPA) system activity and stress during pregnancy: Effects on gestational age and infant's anthropometric measures at birth. *Psychoneuroendocrinology.* 2018;94:152-161.

29. Horsch A, Kang JS, Vial Y, et al. Stress exposure and psychological stress responses are related to glucose concentrations during pregnancy. *Br J Health Psychol.* 2016;21(3):712-729.

30. Kofman YB, Eng ZE, Busse D, et al. Cortisol reactivity and depressive symptoms in pregnancy: The moderating role of perceived social support and neuroticism. *Biol Psychol.* 2019;147:107656.

31. Obel C, Hedegaard M, Henriksen TB, et al. Stress and salivary cortisol during pregnancy. *Psychoneuroendocrinology.* 2005;30(7):647-656.

32. Rothenberger SE, Moehler E, Reck C, et al. Prenatal stress: course and interrelation of emotional and physiological stress measures. *Psychopathology.* 2011;44(1):60-67.

33. Simon CD, Adam EK, Holl JL, et al. Prenatal Stress and the Cortisol Awakening Response in African-American and Caucasian Women in the Third Trimester of Pregnancy. *Maternal and child health journal.* 2016;20(10):2142-2149.

34. Smew AI, Hedman AM, Chiesa F, et al. Limited association between markers of stress during pregnancy and fetal growth in 'Born into Life', a new prospective birth cohort. *Acta Paediatr.* 2018.

35. Thayer ZM, Kuzawa CW. Ethnic discrimination predicts poor self-rated health and cortisol in pregnancy: insights from New Zealand. *Soc Sci Med.* 2015;128:36-42.

36. Valladares E, Peña R, Ellsberg M, et al. Neuroendocrine response to violence during pregnancy--impact on duration of pregnancy and fetal growth. *Acta Obstet Gynecol Scand.* 2009;88(7):818-823.

37. Bolten MI, Wurmser H, Buske-Kirschbaum A, et al. Cortisol levels in pregnancy as a psychobiological predictor for birth weight. *Archives of women's mental health.* 2011;14(1):33-41.

38. Kivlighan KT, DiPietro JA, Costigan KA, et al. Diurnal rhythm of cortisol during late pregnancy: associations with maternal psychological well-being and fetal growth. *Psychoneuroendocrinology.* 2008;33(9):1225-1235.

39. Petraglia F, Hatch MC, Lapinski R, et al. Lack of effect of psychosocial stress on maternal corticotropin-releasing factor and catecholamine levels at 28 weeks' gestation. *J Soc Gynecol Investig.* 2001;8(2):83-88.

40. Spicer J, Werner E, Zhao Y, et al. Ambulatory assessments of psychological and peripheral stress-markers predict birth outcomes in teen pregnancy. *J Psychosom Res.* 2013;75(4):305-313.

41. Wadhwa PD, Dunkel-Schetter C, Chicz-DeMet A, et al. Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. *Psychosom Med.* 1996;58(5):432-446.

42. *Centers for Disease Control and Prevention (US); National Center for Chronic Disease Prevention and Health Promotion (US); Office on Smoking and Health (US). How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General.* Atlanta (GA), US2010.

43. *IARC Working Group on the Evaluation of Carcinogenic Risk to Humans. Tobacco Smoke and Involuntary Smoking.* Lyon, France: International Agency for Research on Cancer;2004. 83.

44. Gunnerbeck A, Raaschou P, Cnattingius S, et al. Maternal snuff use and cotinine in late pregnancy-A validation study. *Acta Obstet Gynecol Scand.* 2018;97(11):1373-1380.

45. Cnattingius S. The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco.* 2004;6 Suppl 2:S125-140.

46. Murin S, Rafii R, Bilello K. Smoking and smoking cessation in pregnancy. *Clin Chest Med.* 2011;32(1):75-91, viii.

47. *Graviditeter, förlossningar och nyfödda barn – Medicinska födelseregistret 1973–2014 – Assisterad befruktning 1991–2013.* Stockholm, Sweden: The Board of Health and Welfare;2015.

48. Humphrey G, Rossen F, Walker N, et al. Parental smoking during pregnancy: findings from the Growing Up in New Zealand cohort. *N Z Med J.* 2016;129(1442):60-74.

49. Phung HN, Bauman AE, Young L, et al. Ecological and individual predictors of maternal smoking behaviour. Looking beyond individual socioeconomic predictors at the community setting. *Addict Behav.* 2003;28(7):1333-1342.

50. Schoenaker D, Ploubidis GB, Goodman A, et al. Factors across the life course predict women's change in smoking behaviour during pregnancy and in midlife: results from the National Child Development Study. *J Epidemiol Community Health.* 2017;71(12):1137-1144.

51. Castles A, Adams EK, Melvin CL, et al. Effects of smoking during pregnancy. Five meta-analyses. *Am J Prev Med.* 1999;16(3):208-215.

52. Neuman A, Hohmann C, Orsini N, et al. Maternal smoking in pregnancy and asthma in preschool children: a pooled analysis of eight birth cohorts. *Am J Respir Crit Care Med.* 2012;186(10):1037-1043.

53. Ferrante G, Antona R, Malizia V, et al. Smoke exposure as a risk factor for asthma in childhood: a review of current evidence. *Allergy Asthma Proc.* 2014;35(6):454-461.

54. Silvestri M, Franchi S, Pistorio A, et al. Smoke exposure, wheezing, and asthma development: a systematic review and meta-analysis in unselected birth cohorts. *Pediatr Pulmonol.* 2015;50(4):353-362.

55. Accordini S, Janson C, Svanes C, et al. The role of smoking in allergy and asthma: lessons from the ECRHS. *Current allergy and asthma reports.* 2012;12(3):185-191.

56. Baba S, Wikstrom AK, Stephansson O, et al. Influence of snuff and smoking habits in early pregnancy on risks for stillbirth and early neonatal mortality. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco.* 2014;16(1):78-83.

57. Wikstrom AK, Cnattingius S, Stephansson O. Maternal use of Swedish snuff (snus) and risk of stillbirth. *Epidemiology.* 2010;21(6):772-778.

58. Baba S, Wikstrom AK, Stephansson O, et al. Influence of smoking and snuff cessation on risk of preterm birth. *Eur J Epidemiol.* 2012;27(4):297-304.

59. England LJ, Levine RJ, Mills JL, et al. Adverse pregnancy outcomes in snuff users. *Am J Obstet Gynecol.* 2003;189(4):939-943.

60. Wikstrom AK, Cnattingius S, Galanti MR, et al. Effect of Swedish snuff (snus) on preterm birth. *BJOG.* 2010;117(8):1005-1010.

61. Juarez SP, Merlo J. The effect of Swedish snuff (snus) on offspring birthweight: a sibling analysis. *PLoS One.* 2013;8(6):e65611.

62. Baba S, Wikstrom AK, Stephansson O, et al. Changes in snuff and smoking habits in Swedish pregnant women and risk for small for gestational age births. *BJOG.* 2013;120(4):456-462.

63. Gunnerbeck A, Wikstrom AK, Bonamy AK, et al. Relationship of maternal snuff use and cigarette smoking with neonatal apnea. *Pediatrics.* 2011;128(3):503-509.

64. Gunnerbeck A, Edstedt Bonamy AK, Wikstrom AK, et al. Maternal snuff use and smoking and the risk of oral cleft malformations--a population-based cohort study. *PLoS One.* 2014;9(1):e84715.

65. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths.

Modifications recommended by FIGO as amended October 14, 1976. *Acta Obstet Gynecol Scand.* 1977;56(3):247-253.

66. ACOG Committee Opinion No 579: Definition of term pregnancy. *Obstet Gynecol.* 2013;122(5):1139-1140.

67. Kramer MS, Papageorghiou A, Culhane J, et al. Challenges in defining and classifying the preterm birth syndrome. *Am J Obstet Gynecol.* 2012;206(2):108-112.

68. Zhang X, Kramer MS. Variations in mortality and morbidity by gestational age among infants born at term. *J Pediatr.* 2009;154(3):358-362, 362.e351.

69. Vogel JP, Chawanpaiboon S, Moller AB, et al. The global epidemiology of preterm birth. *Best Pract Res Clin Obstet Gynaecol.* 2018;52:3-12.

70. *Levels & Trends in Child Mortality, Report 2019.* UN Inter-agency Group for Child Mortality Estimation;2019.

71. Brodsky D, Christou H. Current concepts in intrauterine growth restriction. *J Intensive Care Med.* 2004;19(6):307-319.

72. Faa G, Manchia M, Pintus R, et al. Fetal programming of neuropsychiatric disorders. *Birth defects research Part C, Embryo today : reviews.* 2016;108(3):207-223.

73. Ortqvist AK, Lundholm C, Carlstrom E, et al. Familial factors do not confound the association between birth weight and childhood asthma. *Pediatrics.* 2009;124(4):e737-743.

74. Abraham M, Alramadhan S, Iniguez C, et al. A systematic review of maternal smoking during pregnancy and fetal measurements with meta-analysis. *PLoS One.* 2017;12(2):e0170946.

75. Hogberg L, Lundholm C, Cnattingius S, et al. Birthweight discordant female twins and their offspring: is the intergenerational influence on birthweight due to genes or environment? *Hum Reprod.* 2013;28(2):480-487.

76. Lima SAM, El Dib RP, Rodrigues MRK, et al. Is the risk of low birth weight or preterm labor greater when maternal stress is experienced during pregnancy? A systematic review and meta-analysis of cohort studies. *PLoS One.* 2018;13(7):e0200594.

77. Accortt EE, Cheadle AC, Dunkel Schetter C. Prenatal depression and adverse birth outcomes: an updated systematic review. *Maternal and child health journal.* 2015;19(6):1306-1337.

78. Grigoriadis S, VonderPorten EH, Mamisashvili L, et al. The impact of maternal depression during pregnancy on perinatal outcomes: a systematic review and meta-analysis. *J Clin Psychiatry.* 2013;74(4):e321-341.

79. Grote NK, Bridge JA, Gavin AR, et al. A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. *Arch Gen Psychiatry.* 2010;67(10):1012-1024.

80. Ding XX, Wu YL, Xu SJ, et al. Maternal anxiety during pregnancy and adverse birth outcomes: a systematic review and meta-analysis of prospective cohort studies. *J Affect Disord.* 2014;159:103-110.

81. Grigoriadis S, Graves L, Peer M, et al. Maternal Anxiety During Pregnancy and the Association With Adverse Perinatal Outcomes: Systematic Review and Meta-Analysis. *J Clin Psychiatry.* 2018;79(5).

82. Warland J, Dorrian J, Morrison JL, et al. Maternal sleep during pregnancy and poor fetal outcomes: A scoping review of the literature with meta-analysis. *Sleep Med Rev.* 2018;41:197-219.

83. Goedhart G, Vrijkotte TG, Roseboom TJ, et al. Maternal cortisol and offspring birthweight: results from a large prospective cohort study. *Psychoneuroendocrinology.* 2010;35(5):644-652.

84. Braithwaite EC, Hill J, Pickles A, et al. Associations between maternal prenatal cortisol and fetal growth are specific to infant sex: findings from the Wirral Child Health and Development Study. *J Dev Orig Health Dis.* 2018:1-7.

85. D'Anna-Hernandez KL, Hoffman MC, Zerbe GO, et al. Acculturation, maternal cortisol, and birth outcomes in women of Mexican descent. *Psychosom Med.* 2012;74(3):296-304.

86. Giesbrecht GF, Campbell T, Letourneau N. Sexually dimorphic adaptations in basal maternal stress physiology during pregnancy and implications for fetal development. *Psychoneuroendocrinology.* 2015;56:168-178.

87. Erickson K, Thorsen P, Chrousos G, et al. Preterm birth: associated neuroendocrine, medical, and behavioral risk factors. *J Clin Endocrinol Metab.* 2001;86(6):2544-2552.

88. Stewart CP, Oaks BM, Laugero KD, et al. Maternal cortisol and stress are associated with birth outcomes, but are not affected by lipid-based nutrient supplements during pregnancy: an analysis of data from a randomized controlled trial in rural Malawi. *BMC Pregnancy Childbirth.* 2015;15:346.

89. Hompes T, Vrieze E, Fieuws S, et al. The influence of maternal cortisol and emotional state during pregnancy on fetal intrauterine growth. *Pediatr Res.* 2012;72(3):305-315.

90. Diego MA, Field T, Hernandez-Reif M, et al. Prenatal depression restricts fetal growth. *Early Hum Dev.* 2009;85(1):65-70.

91. Ramakrishnan U, Martorell R, Schroeder DG, et al. Role of intergenerational effects on linear growth. *J Nutr.* 1999;129(2S Suppl):544s-549s.

92. Cheong JN, Wlodek ME, Moritz KM, et al. Programming of maternal and offspring disease: impact of growth restriction, fetal sex and transmission across generations. *J Physiol.* 2016;594(17):4727-4740.

93. Breton CV, Siegmund KD, Joubert BR, et al. Prenatal tobacco smoke exposure is associated with childhood DNA CpG methylation. *PLoS One.* 2014;9(6):e99716.

94. Joubert BR, Felix JF, Yousefi P, et al. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. *Am J Hum Genet.* 2016;98(4):680-696.

95. Monasso GS, Jaddoe VWV, de Jongste JC, et al. Timing- and Dose-Specific Associations of Prenatal Smoke Exposure With Newborn DNA Methylation. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco.* 2020;22(10):1917-1922.

96. Hypponen E, Smith GD, Power C. Effects of grandmothers' smoking in pregnancy on birth weight: intergenerational cohort study. *BMJ.* 2003;327(7420):898.

97. Pembrey M, Northstone K, Gregory S, et al. Is the growth of the child of a smoking mother influenced by the father's prenatal exposure to tobacco? A hypothesis generating longitudinal study. *BMJ Open.* 2014;4(7):e005030.

98. Miller LL, Pembrey M, Davey Smith G, et al. Is the growth of the fetus of a non-smoking mother influenced by the smoking of either grandmother while pregnant? *PLoS One.* 2014;9(2):e86781.

99. Rillamas-Sun E, Harlow SD, Randolph JF, Jr. Grandmothers' smoking in pregnancy and grandchildren's birth weight: comparisons by grandmother birth cohort. *Maternal and child health journal.* 2014;18(7):1691-1698.

100. Misra DP, Astone N, Lynch CD. Maternal smoking and birth weight: interaction with parity and mother's own in utero exposure to smoking. *Epidemiology.* 2005;16(3):288-293.

101. Ding M, Yuan C, Gaskins AJ, et al. Smoking during pregnancy in relation to grandchild birth weight and BMI trajectories. *PLoS One.* 2017;12(7):e0179368.

102. Shen Y, Zhang H, Jiang Y, et al. Maternal Birth Weight and BMI Mediate the Transgenerational Effect of Grandmaternal BMI on Grandchild's Birth Weight. *Obesity (Silver Spring, Md).* 2020.

103. *The Global Asthma Report 2014.* Auckland, New Zealand: Global Asthma Network;2014.

104. Bjerg A, Sandstrom T, Lundback B, et al. Time trends in asthma and wheeze in Swedish children 1996-2006: prevalence and risk factors by sex. *Allergy.* 2010;65(1):48-55.

105. Almqvist C, Pershagen G, Wickman M. Low socioeconomic status as a risk factor for asthma, rhinitis and sensitization at 4 years in a birth cohort. *Clin Exp Allergy.* 2005;35(5):612-618.

106. Castro-Rodriguez JA, Forno E, Rodriguez-Martinez CE, et al. Risk and Protective Factors for Childhood Asthma: What Is the Evidence? *The journal of allergy and clinical immunology In practice.* 2016;4(6):1111-1122.

107. Duijts L. Fetal and infant origins of asthma. *Eur J Epidemiol.* 2012;27(1):5-14.

108. Gong T, Lundholm C, Rejno G, et al. Parental socioeconomic status, childhood asthma and medication use--a population-based study. *PLoS One.* 2014;9(9):e106579.

109. Krzych-Fałta E, Furmańczyk K, Lisiecka-Biełanowicz M, et al. The effect of selected risk factors, including the mode of delivery, on the development of allergic rhinitis and bronchial asthma. *Postepy dermatologii i alergologii.* 2018;35(3):267-273.

110. Hearn AP, Kent BD, Jackson DJ. Biologic treatment options for severe asthma. *Curr Opin Immunol.* 2020;66:151-160.

111. *Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention.* 2016.

112. Chung KF. Managing severe asthma in adults: lessons from the ERS/ATS guidelines. *Curr Opin Pulm Med.* 2015;21(1):8-15.

113. Sekhon HS, Jia Y, Raab R, et al. Prenatal nicotine increases pulmonary alpha7 nicotinic receptor expression and alters fetal lung development in monkeys. *J Clin Invest.* 1999;103(5):637-647.

114. Sekhon HS, Keller JA, Benowitz NL, et al. Prenatal nicotine exposure alters pulmonary function in newborn rhesus monkeys. *Am J Respir Crit Care Med.* 2001;164(6):989-994.

115. Gilman SE, Gardener H, Buka SL. Maternal smoking during pregnancy and children's cognitive and physical development: a causal risk factor? *Am J Epidemiol.* 2008;168(5):522-531.

116. Freudenberg N, Ruglis J. Reframing school dropout as a public health issue. *Prev Chronic Dis.* 2007;4(4):A107.

117. Lê-Scherban F, Diez Roux AV, Li Y, et al. Does academic achievement during childhood and adolescence benefit later health? *Ann Epidemiol.* 2014;24(5):344-355.

118. Gunnell D, Löfving S, Gustafsson JE, et al. School performance and risk of suicide in early adulthood: follow-up of two national cohorts of Swedish schoolchildren. *J Affect Disord.* 2011;131(1-3):104-112.

119. Kosidou K, Dalman C, Fredlund P, et al. School Performance and the Risk of Suicidal Thoughts in Young Adults: Population-Based Study. *PLoS One.* 2014;9(10):4.

120. Acacio-Claro PJ, Koivusilta LK, Borja JR, et al. Adolescent reserve capacity, socioeconomic status and school achievement as predictors of mortality in Finland - a longitudinal study. *BMC Public Health.* 2017;17(1):980.

121. Bradley RH, Corwyn RF. Socioeconomic status and child development. *Annu Rev Psychol.* 2002;53:371-399.

122. Rabiner DL, Godwin J, Dodge KA. Predicting Academic Achievement and Attainment: The Contribution of Early Academic Skills, Attention Difficulties, and Social Competence. *School Psych Rev.* 2016;45(2):250-267.

123. Fan XT, Chen M. Parental involvement and students' academic achievement: A meta-analysis. *Educ Psychol Rev.* 2001;13(1):1-22.

124. Karbach J, Gottschling J, Spengler M, et al. Parental involvement and general cognitive ability as predictors of domain-specific academic achievement in early adolescence. *Learn Instr.* 2013;23:43-51.

125. Buchmann C, DiPrete TA, McDaniel A. Gender inequalities in education. *Annu Rev Sociol.* 2008;34:319-337.

126. Allotey J, Zamora J, Cheong-See F, et al. Cognitive, motor, behavioural and academic performances of children born preterm: a meta-analysis and systematic review involving 64 061 children. *BJOG.* 2018;125(1):16-25.

127. Lee J, Stankov L. Non-cognitive predictors of academic achievement: Evidence from TIMSS and PISA. *Learn Individ Differ.* 2018;65:50-64.

128. Daley D, Birchwood J. ADHD and academic performance: why does ADHD impact on academic performance and what can be done to support ADHD children in the classroom? *Child Care Health Dev.* 2010;36(4):455-464.

129. Jangmo A, Stalhandske A, Chang Z, et al. Attention-Deficit/Hyperactivity Disorder, School Performance, and Effect of Medication. *J Am Acad Child Adolesc Psychiatry.* 2019;58(4):423-432.

130. Milton B, Whitehead M, Holland P, et al. The social and economic consequences of childhood asthma across the lifecourse: a systematic review. *Child Care Health Dev.* 2004;30(6):711-728.

131. Moonie SA, Sterling DA, Figgs L, et al. Asthma status and severity affects missed school days. *J Sch Health.* 2006;76(1):18-24.

132. Taras H, Potts-Datema W. Childhood asthma and student performance at school. *J Sch Health.* 2005;75(8):296-312.

133. Celano MP, Geller RJ. Learning, school performance, and children with asthma: how much at risk? *J Learn Disabil.* 1993;26(1):23-32.

134. Stores G, Ellis AJ, Wiggs L, et al. Sleep and psychological disturbance in nocturnal asthma. *Arch Dis Child.* 1998;78(5):413-419.

135. Irani F, Barbone JM, Beausoleil J, et al. Is asthma associated with cognitive impairments? A meta-analytic review. *J Clin Exp Neuropsychol.* 2017;39(10):965-978.

136. Nilsson S, Odling M, Andersson N, et al. Does asthma affect school performance in adolescents? Results from the Swedish population-based birth cohort BAMSE. *Pediatr Allergy Immunol.* 2018;29(2):174-179.

137. Holmberg K, Lundholm C, Anckarsater H, et al. Impact of asthma medication and familial factors on the association between childhood asthma and attention-deficit/hyperactivity disorder: a combined twin- and register-based study: Epidemiology of Allergic Disease. *Clin Exp Allergy.* 2015;45(5):964-973.

138. Mogensen N, Larsson H, Lundholm C, et al. Association between childhood asthma and ADHD symptoms in adolescence--a prospective population-based twin study. *Allergy.* 2011;66(9):1224-1230.

139. Schmitt J, Buske-Kirschbaum A, Roessner V. Is atopic disease a risk factor for attention-deficit/hyperactivity disorder? A systematic review. *Allergy.* 2010;65(12):1506-1524.

140. Kozyrskyj AL, Kendall GE, Jacoby P, et al. Association between socioeconomic status and the development of asthma: analyses of income trajectories. *Am J Public Health.* 2010;100(3):540-546.

141. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology.* 1999;10(1):37-48.

142. Krieger N, Davey Smith G. The tale wagged by the DAG: broadening the scope of causal inference and explanation for epidemiology. *Int J Epidemiol.* 2016;45(6):1787-1808.

143. Vandenbroucke JP, Broadbent A, Pearce N. Causality and causal inference in epidemiology: the need for a pluralistic approach. *Int J Epidemiol.* 2016;45(6):1776-1786.

144. D'Onofrio BM, Lahey BB, Turkheimer E, et al. Critical need for family-based, quasi-experimental designs in integrating genetic and social science research. *Am J Public Health.* 2013;103 Suppl 1:S46-55.

145. Sjolander A, Zetterqvist J. Confounders, Mediators, or Colliders: What Types of Shared Covariates Does a Sibling Comparison Design Control For? *Epidemiology.* 2017;28(4):540-547.

146. Frisell T, Öberg S, Kuja-Halkola R, et al. Sibling comparison designs: bias from non-shared confounders and measurement error. *Epidemiology.* 2012;23(5):713-720.

147. Sjolander A, Frisell T, Kuja-Halkola R, et al. Carryover Effects in Sibling Comparison Designs. *Epidemiology.* 2016;27(6):852-858.

148. Richiardi L, Bellocco R, Zugna D. Mediation analysis in epidemiology: methods, interpretation and bias. *Int J Epidemiol.* 2013;42(5):1511-1519.

149. VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. *Annu Rev Public Health.* 2016;37:17-32.

150. VanderWeele TJ. *Explanation in causal inference : methods for mediation and interaction.* New York, USA: Oxford University Press; 2015.

151. Greenland S, Robins JM, Pearl J. Confounding and collapsibility in causal inference. *Stat Sci.* 1999;14(1):29-46.

152. Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods.* 2013;18(2):137-150.

153. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Epidemiology.* 2007;18(6):800-804.

154. Vandenbroucke JP, von Elm E, Altman DG, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Epidemiology.* 2007;18(6):805-835.

155. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, et al. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol.* 2009;24(11):659-667.

156. Axelsson O. The Swedish medical birth register. *Acta Obstet Gynecol Scand.* 2003;82(6):491-492.

157. *The Swedish Medical Birth Register - A summary of content and quality.* Stockholm: Swedish National Board of Health and Welfare, Center of Epidemiology;2003.

158. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health.* 2011;11:450.

159. Ludvigsson JF, Almqvist C, Bonamy AK, et al. Registers of the Swedish total population and their use in medical research. *Eur J Epidemiol.* 2016;31(2):125-136.

160. Ludvigsson JF, Svedberg P, Olen O, et al. The longitudinal integrated database for health insurance and labour market studies (LISA) and its use in medical research. *Eur J Epidemiol.* 2019.

161. Ekbom A. The Swedish Multi-generation Register. *Methods Mol Biol.* 2011;675:215-220.

162. Nordin M, Nordin S. Psychometric evaluation and normative data of the Swedish version of the 10-item perceived stress scale. *Scand J Psychol.* 2013;54(6):502-507.

163. Yokokura A, Silva A, Fernandes JKB, et al. Perceived Stress Scale: confirmatory factor analysis of the PSS14 and PSS10 versions in two samples of pregnant women from the BRISA cohort. *Cad Saude Publica.* 2017;33(12):e00184615.

164. Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology.* 2009;34(10):1423-1436.

165. Marsál K, Persson PH, Larsen T, et al. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr.* 1996;85(7):843-848.

166. Ortqvist AK, Lundholm C, Wettermark B, et al. Validation of asthma and eczema in population-based Swedish drug and patient registers. *Pharmacoepidemiol Drug Saf.* 2013;22(8):850-860.

167. Larsson H, Ryden E, Boman M, et al. Risk of bipolar disorder and schizophrenia in relatives of people with attention-deficit hyperactivity disorder. *Br J Psychiatry.* 2013;203(2):103-106.

168. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med.* 2017;167(4):268-274.

169. Smith LH, VanderWeele TJ. Mediational E-values: Approximate Sensitivity Analysis for Unmeasured Mediator-Outcome Confounding. *Epidemiology.* 2019;30(6):835-837.

170. Lambert PC, Royston P. Further development of flexible parametric models for survival analysis. *Stata J.* 2009;9(2):265-290.

171. Cohen J. A power primer. *Psychol Bull.* 1992;112(1):155-159.

172. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA.* 2013;310(20):2191-2194.

173. Lundholm C, Gunnerbeck A, D'Onofrio BM, et al. Smoking and snuff use in pregnancy and the risk of asthma and wheeze in pre-schoolchildren-A population-based register study. *Clin Exp Allergy.* 2020;50(5):597-608.

174. Lundholm C, Brew BK, D'Onofrio BM, et al. Asthma and subsequent school performance at age 15-16 years: A Swedish population-based sibling control study. *Sci Rep.* 2020;10(1):7661.

175. Rothman KJ. *Modern epidemiology.* Third edition ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.

176. Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. *Epidemiology.* 2004;15(5):615-625.

177. Mattsson K, Kallen K, Rignell-Hydbom A, et al. Cotinine Validation of Self-Reported Smoking During Pregnancy in the Swedish Medical Birth Register. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco.* 2016;18(1):79-83.

178. Hasanjanzadeh P, Faramarzi M. Relationship between Maternal General and Specific-Pregnancy Stress, Anxiety, and Depression Symptoms and Pregnancy Outcome. *Journal of clinical and diagnostic research : JCDR.* 2017;11(4):Vc04-vc07.

179. Liou SR, Wang P, Cheng CY. Effects of prenatal maternal mental distress on birth outcomes. *Women and birth : journal of the Australian College of Midwives.* 2016;29(4):376-380.

180. Rondo PH, Ferreira RF, Nogueira F, et al. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *Eur J Clin Nutr.* 2003;57(2):266-272.

181. Khashan AS, Everard C, McCowan LM, et al. Second-trimester maternal distress increases the risk of small for gestational age. *Psychol Med.* 2014;44(13):2799-2810.

182. Lau Y. The effect of maternal stress and health-related quality of life on birth outcomes among Macao Chinese pregnant women. *J Perinat Neonatal Nurs.* 2013;27(1):14-24.

183. Vardavas CI, Hohmann C, Patelarou E, et al. The independent role of prenatal and postnatal exposure to active and passive smoking on the development of early wheeze in children. *Eur Respir J.* 2016;48(1):115-124.

184. Gilliland FD, Li YF, Peters JM. Effects of maternal smoking during pregnancy and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med.* 2001;163(2):429-436.

185. Sherriff A, Peters TJ, Henderson J, et al. Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3(1/2) years. *Int J Epidemiol.* 2001;30(6):1473-1484.

186. Duijts L, Jaddoe VWV, van der Valk RJP, et al. Fetal exposure to maternal and paternal smoking and the risks of wheezing in preschool children: the Generation R Study. *Chest.* 2012;141(4):876-885.

187. Hagendorens MM, Bridts CH, Lauwers K, et al. Perinatal risk factors for sensitization, atopic dermatitis and wheezing during the first year of life (PIPO study). *Clin Exp Allergy.* 2005;35(6):733-740.

188. Tanaka K, Miyake Y, Sasaki S, et al. Maternal smoking and environmental tobacco smoke exposure and the risk of allergic diseases in Japanese infants: the Osaka Maternal and Child Health Study. *J Asthma.* 2008;45(9):833-838.

189. Lannero E, Wickman M, Pershagen G, et al. Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). *Respir Res.* 2006;7:3.

190. Fleming M, Fitton CA, Steiner MFC, et al. Educational and health outcomes of children treated for asthma: Scotland-wide record linkage study of 683,716 children. *Eur Respir J.* 2019.

191. Fowler MG, Davenport MG, Garg R. School functioning of US children with asthma. *Pediatrics.* 1992;90(6):939-944.

192. Kim SY, Kim MS, Park B, et al. Allergic rhinitis, atopic dermatitis, and asthma are associated with differences in school performance among Korean adolescents. *PLoS One.* 2017;12(2):e0171394.

193. Kohen DE. Asthma and school functioning. *Health Rep.* 2010;21(4):35-45.

194. Liberty KA, Pattemore P, Reid J, et al. Beginning school with asthma independently predicts low achievement in a prospective cohort of children. *Chest.* 2010;138(6):1349-1355.

195. Ruijsbroek A, Wijga AH, Kerkhof M, et al. The development of socio-economic health differences in childhood: results of the Dutch longitudinal PIAMA birth cohort. *BMC Public Health.* 2011;11:225.

196. Tsakiris A, Iordanidou M, Paraskakis E, et al. The presence of asthma, the use of inhaled steroids, and parental education level affect school performance in children. *Biomed Res Int.* 2013;2013:762805.

197. Brew BK, Soderberg J, Lundholm C, et al. Academic achievement of adolescents with asthma or atopic disease. *Clin Exp Allergy.* 2019.

198. Gutstadt LB, Gillette JW, Mrazek DA, et al. Determinants of school performance in children with chronic asthma. *Am J Dis Child.* 1989;143(4):471-475.

199. Lindgren S, Lokshin B, Stromquist A, et al. Does asthma or treatment with theophylline limit children's academic performance? *N Engl J Med.* 1992;327(13):926-930.

200. Moonie S, Sterling DA, Figgs LW, et al. The relationship between school absence, academic performance, and asthma status. *J Sch Health.* 2008;78(3):140-148.

201. Rietveld S, Colland VT. The impact of severe asthma on schoolchildren. *J Asthma.* 1999;36(5):409-417.

202. Ross S, Godden D, McMurray D, et al. Social effects of wheeze in childhood: a 25 year follow up. *BMJ.* 1992;305(6853):545-548.

203. Miyazaki C, Koyama M, Ota E, et al. Allergic diseases in children with attention deficit hyperactivity disorder: a systematic review and meta-analysis. *BMC Psychiatry.* 2017;17(1):120.

204. Russell E, Koren G, Rieder M, et al. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. *Psychoneuroendocrinology.* 2012;37(5):589-601.

205. Wosu AC, Valdimarsdóttir U, Shields AE, et al. Correlates of cortisol in human hair: implications for epidemiologic studies on health effects of chronic stress. *Ann Epidemiol.* 2013;23(12):797-811.e792.

206. Hedman L, Backman H, Stridsman C, et al. Predictors of electronic cigarette use among Swedish teenagers: a population-based cohort study. *BMJ Open.* 2020;10(12):e040683.

207. Jones RD, Asare M, Lanning B. A Retrospective Cross-Sectional Study on the Prevalence of E-cigarette Use Among College Students. *J Community Health.* 2021;46(1):195-202.

208. Strongin RM. E-Cigarette Chemistry and Analytical Detection. *Annu Rev Anal Chem (Palo Alto Calif).* 2019;12(1):23-39.

209. Kaplan A, Price D. Treatment Adherence in Adolescents with Asthma. *Journal of asthma and allergy.* 2020;13:39-49.